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RELATIONSHIPS AMONG PLANT CALCIFICATION, PLANT FORM AND HERBIVORE MODE OF FEEDING IN MARINE PLANT-HERBIVORE INTERACTIONS

by

(C)

DIANNA K. PADILLA

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

IN

ZOOLOGY

EDMONTON, ALBERTA

SPRING 1987

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled RELATIONSHIPS AMONG PLANT CALCIFICATION, PLANT FORM AND HERBIVORE MODE OF FEEDING IN MARINE PLANT-HERBIVORE INTERACTIONS submitted by DIANNA K. PADILLA in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY in ZOOLOGY

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Date..January 29 1987..................

This thesis is dedicated to Carmella M. Genova,
and "those poor little animals without any eyes"
that mad her laugh until she cried.

ABSTRACT

Mechanical tests were made of models that predict that algal form and calcification act primarily as structural defenses of marine algae by reducing algal susceptibility to herbivores. The mechanical resistance of a variety of marine algae (tropical and temperate) to herbivores of two important feeding types, rasping herbivores (docoglossan limpets) and a biting herbivore (an herbivorous crab), was measured. In all cases the results did not support the predictions of the models.

I suggest that another factor, the probability of subsequent tissue loss due to herbivore damage, may play an important role in the impact of herbivores on the algae they consume. For some algal species, secondary tissue loss may greatly exceed primary loss to herbivores. Calcification and other potential "structural defenses" such as thallus form may play a more important role in minimizing this secondary tissue loss than in preventing herbivores from removing tissue as has been suggested in the past.

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Chapter I. General Introduction.

Marine macrophytic algae vary considerably in thallus form not only among species but also within a single species in different environments or at different life-history stages (Mathieson et al. 1981). Recently, several reviews have been made of marine plant-herbivore interactions (Lubchenco and Gaines 1981, Gaines and Lubchenco 1982, Hawkins and Hartnoll 1983). These reviewers have suggested causal links between herbivore and plant distributions and correlations between the relative abundance of different marine plant growth forms and other environmental factors. These authors suggested that a) plant form, b) mode of feeding of the herbivores, and c) both chemical and structural defenses of plants, all influence the resistance of plants to herbivores. However, they also indicated that there is insufficient empirical data to draw general conclusions about the relative importance of these factors in determining plant distributions.

In an effort to construct a generalized approach to marine plant-herbivore interactions, Lubchenco and Gaines (1981) formulated a conditional probability model to determine the potential impact of herbivores on marine plants. This model included three terms: 1) the probability that a plant will be encountered by an herbivore, 2) the probability that the herbivore will eat a plant given it is encountered, and 3) the expected decline in fitness, relative to the rest of the population, due to the consumption of tissue by the herbivore.

Given this model, the potential defenses of plants against their herbivores would include avoiding herbivores either temporally or spatially (by alterations in the timing of life-history stages or microhabitat use), as well as preventing consumption of the plant once encountered. Structural and chemical defenses were considered to act primarily by reducing the probability that an herbivore would eat part of a plant.

Gaines and Lubchenco divided the marine macrophytes into seven forms: sheet-like or tubular, filamentous, fleshy bladed, fleshy branched, erect calcareous, fleshy crustose and calcareous crustose. They found that the relative abundances of most of these groups varied with latitude, and differed between the east and west coasts of North America at the same latitudes. In most cases these patterns were correlated with herbivore abundance and diversity. On shores with a greater abundance and diversity of herbivores, which have been interpreted as having greater intensity of herbivory, plant forms considered herbivore-resistant were relatively more abundant. These herbivore-resistant plants included the calcified plants, both crustose and erect, fleshy crustose plants and noncalcified branched plants (Gaines and Lubchenco 1982). Hawkins and Hartnoll (1983) noted similar patterns and also attributed herbivore resistance to the same algal forms.

Littler and Littler (1980) and Steneck and Watling (1982) constructed verbal models which, in addition to predicting relationships among other aspects of marine plant biology (productivity, calorific content, community structure), also make predictions about algal resistance to herbivores based on plant form. Unfortunately, both of these models ignore the role of anti-herbivore chemicals, and thus assume that plant form plays an overriding influence on plant resistance to herbivores. The Littler and Littler model of functional-form groupings for marine algae predicts primary productivity, resistance to grazers, calorific content and successional status on the basis of plant form. This model suggests that persistant forms which allocate resources for environmental resistance, interference competition or anti-herbivore defenses do so at the cost of rate of primary production. They also categorize the algae into morphological groups, and these groupings are used to predict algal resistance to herbivores and thallus mechanical reistance to herbivore damage (Table I-1).

Steneck and Watling (1982) constructed a similar model based on thallus size, morphology, and resistance to scratching, to place algae into seven functional groups. They recognized that an alga can change groups ontogenetically, and that different parts of the thallus could be considered in different groups. They use these groupings to predict algal susceptibility to molluscan grazers (Table I-1) and algal community structure.

All of these ideas and patterns have been synthesized into a general view of algal morphology suggesting that plant form and calcification act primarily to increase algal mechanical resistance to herbivore damage (Littler and Littler 1980, Johansen 1981, Lubchenco and Gaines 1981, Gaines and Lubchenco 1982, Steneck and Watling 1982, Hawkins and Hartnoll 1983, Littler, et al. 1983; Table I-1). Although this view is appealing and has gained widespread agreement, very few attempts have been made to directly assess the mechanical resistance of marine plants to grazing by herbivores.

I measured the mechanical resistance of a variety of marine algae (both tropical and temperate) to herbivores of two important feeding types, rasping herbivores (docoglossan limpets, Chapters II and III) and a biting herbivore (an herbivorous crab, Chapter IV). By measuring the mechanical resistance of plants to herbivores directly, rather than by using herbivore preferences, one eliminates potential confounding factors such as feeding stimulants, feeding deterrents, or the nutritional quality of the plant.

I suggest that another factor be incorporated into the Lubchenco and Gaines (1981) model of the impact of herbivores on plants they consume: the probability of subsequent tissue loss precipitated by herbivore damage. For some algal species, secondary tissue loss may greatly exceed primary loss to herbivores (Black 1974, Santelices et al. 1980, Johnson and Mann 1986). I suggest that calcification and other potential "structural defenses" such as thallus form may play a more important role in minimizing this secondary tissue loss than in preventing herbivores from removing tissue as has been suggested in the past. Plants could minimize secondary loss in several ways, including having large breaking strengths or by preventing tissue damage from subsequently weakening the structure of the plant. I conducted experiments to test whether calcification could increase plant structural strength and prevent the propagation of damage in marine macrophytes.

The Lubchenco and Gaines model also assumes that herbivory and tissue loss have negative effects on plants, which translate directly to a decline in fitness relative to undamaged plants. Other researchers have suggested that certain levels of herbivory and tissue loss may be beneficial to plants by removing epiphytes (Paine 1980, Steneck 1982, 1983, 1986) or by decreasing the probability of a large plant being dislodged (Black 1974). For long-lived plants, herbivory or preiodic tissue loss may translate to increases in relative fitness. Therefore, I also suggest that the Lubchenco and Gaines model be made more general, and consider the change, positive or negative, in relative fitness of damaged plants as compared to undamaged plants.

Table I-1. Predictions of three models regarding the structural resistance of macroalgae to herbivores.

FUNCTIONAL FORM (1)	FUNCTIONAL GROUP (2)	CALCIFICATION (3)	SUSCEPTIBILITY
SHEET	FILAMENTOUS	NON-CALCIFIED	MOST SUSCEPTIBLE
FILAMENTOUS	FOLIOSE		
COARSELY BRANCHED	CORTICATED		
THICK LEATHERY	LEATHERY	LIGHTLY CALCIFIED	
JOINTED CALCAREOUS	ARTICULATED CALCAREOUS		
CRUSTOSE	CRUSTOSE CORALLINE	HEAVILY CALCIFIED	LEAST SUSCEPTIBLE

(1) Littler and Littler 1980
(2) Steneck and Watling 1982
(3) Johansen 1981

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Chapter II. Structural resistance of algae to herbivores: a biomechanical approach[1]

INTRODUCTION

Algal form and herbivore mode of feeding have been considered to be major factors that influence plant susceptibility to herbivore damage. Several researchers have placed algae into functional groups based on thallus form, and predicted that these groups would exhibit differential susceptibility to herbivores (Littler and Littler 1980; Steneck and Watling 1982).

Limpets are common intertidal herbivores. They have a docoglossan type radula. A docoglossan radula is characterized by few teeth per row, with the teeth rigidly fixed to the radular ribbon rather than articulating with it (Fretter and Graham 1962). Teeth are constantly being produced posteriorly and shed anteriorly (Figure II-1). Radular tooth number and shape differ greatly among limpets (Fretter and Graham 1962). Their diets also vary substantially. Some limpets are specialists and eat only a few species of algae, while others are generalists and have broad diets (Branch 1981).

The docoglossan radula functions differently from the radular types of other herbivorous gastropods (Fretter and Graham 1962). In the docoglossa (families Patellidae, Acmaeidae, and Lepetidae), the buccal mass and the teeth move together, without any pivoting of one on the other. Numerous rows of teeth are applied to the substrate simultaneously and the whole structure is moved as a unit. This action scores the substratum with a number of parallel lines, indicating the absence of tooth rotation (for a more complete description of gastropod feeding see Fretter and Graham 1962).

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[1] A version of this chapter has been published. D.K. Padilla 1985. Marine Biology. 90: 103-109.

The radular teeth of limpets are generally mineralized with iron and/or silica. These minerals are very hard (5.0 to 6.5 on Mohs scale of hardness; Runham et al. 1969), and therefore allow the limpets to eat endolithic or calcified algae whose hardness is less than this (the hardness of calcite is 3.0).

This study involved several aspects of feeding in two limpet species: Acmaea mitra, a specialist herbivore which preys only on encrusting, calcified algae in the family Corallinaceae, and Tectura (=Notoacmea) scutum, a generalist herbivore. These species are common in the mid and low intertidal region (+1.3 m to -1.0 m; 0= mean lower low water, U.S. tidal datum) of the Pacific coast of North America. Both limpets have docoglossan radulae and therefore feed in a similar fashion. Both have teeth mineralized with iron and silica (Lowenstam 1962, 1981; Padilla, unpublished data).

Initially, the feeding mode was observed to confirm the description of docoglossan feeding by Fretter and Graham (1962). I then determined the forces required for radulae of these limpets to remove tissue from three algal species with different thallus forms. The algae tested were: (1) Hedophyllum sessile, a leathery, brown alga, (2) Iridaea cordata, a thin, bladed red alga, and (3) Pseudolithophyllum whidbeyensi, an encrusting, calcified red alga. Finally, I attempted to determine some of the parameters of tooth morphology that are important in removing plant tissue. In particular, the role of the cutting angles of teeth was examined by the use of scale models, comparing this biological system to what is known about abrasive tools in machining.

This information was used to test some of the predictions made by functional form (Littler and Littler 1980) and functional group (Steneck and Watling 1982) models which predict algal structural resistance to herbivores on the basis of thallus form. These two models predict that the encrusting coralline alga would be the most resistant to herbivores, and that the bladed red alga the least resistant.

MATERIALS AND METHODS

I observed feeding with a video camera focused through a dissecting microscope. A limpet was placed on a glass slide that had been lightly coated with diatoms to induce feeding. The slide was then inverted over a dish filled with sea water, completely submerging the limpet.

The forces required to remove tissue from the algae were determined as follows. Radulae from freshly collected limpets were cleaned of tissue and kept moist until used. Isolated radulae were attached to a Plexiglas support (2.2 mm wide) that placed the radula in feeding position. The anterior most portion of each radula was attached with a cyanoacrylate adhesive, positioning four to six rows of teeth on the working surface of the support. The support was then attached to a beam placing the radular ribbon parallel to an alga placed beneath it (Figure II-2A). The beam was counterbalanced in such a way that the radula and support placed no force on the alga. Various loads (= normal forces) were applied to the radula by attaching weights with dual adhesive tape to the beam directly above the radula.

The alga being tested was attached at one end with a cyanoacrylate glue to a glass slide. The glass slide rode on tracks of ball bearings, thus minimizing friction (Figure II-2B). The slide was attached to a force transducer mounted on a micromanipulator. Thus, when the alga was pulled past the radula with the micromanipulator, the force required to do so (= lateral force) was determined.

A linearly variable differential transformer (LVDT, Pickering and Co., Inc. model 7307 W3-AO) was mounted on the micromanipulator to determine the velocity at which the alga was moved. The LVDT measures the distance the alga travels, and using the chart speed of the recorder as a time base one can calculate the velocity as the slope of the charted displacement curve. These velocities were matched to those of radulae in live limpets when feeding. At least four replicate trials each were made for a range of normal forces (0.049 N to 0.400 N) with radulae from both limpet species on all three algal species.

Algae and radulae were kept wet, but not submerged during all trials (they were removed from the water immediately before each trial). At the end of each test, pieces of the bladed algae were frozen and sectioned with a cryostat. Cross sections were examined to determine if any tissue had been removed by the radulae. The crustose coralline algae were air dried and examined with a scanning electron microscope (SEM) to determine if any tissue had been removed by the radulae. These procedures also allowed the size and characteristics of the marks made by the radulae to be determined.

Size, tooth angles and relative proportions of the radular teeth of both Acmaea mitra and Tectura scutum were determined from SEM micrographs. Camera lucida drawings were used to obtain profiles of the teeth. This information was used to construct large scale models of radular teeth from Plexiglas. Models were of equal width, so that only tooth shape varied. The models were then scraped across a smooth plaster of Paris surface to determine the effect of varying tooth angle. The effect was determined by the removal of plaster of Paris.

Finally, the radula of Acmaea mitra was attached in an orientation backwards from the normal feeding position, and the algae pulled past the radula in the same fashion as described above. This manipulation maintained all of the properties of this radula constant except for the tooth angles. Comparing these results with those of the radula in feeding position is an additional test of the importance of tooth angles.

RESULTS

Force required to remove tissue

Video observations of feeding in Tectura scutum confirmed the reports of feeding behavior in the literature (Fretter and Graham 1962). Acmaea mitra would not feed under these conditions. However, the marks made by the radulae of A. mitra in the force experiments closely resembled the marks made by these limpets when feeding in the field and under laboratory conditions (Padilla, personal observation).

For all algae except the crustose coralline, the radula of Acmaea mitra required more normal force to remove tissue than that of Tectura scutum. However, for both limpets, the rank order of vulnerability of the algae was the same (Figures II-3, II-4). The crustose coralline required the least amount of normal force to remove tissue, the leathery brown blade required the second most, and the thin red blade required the most force.

Tooth structure

Acmaea mitra has six similarly-shaped teeth per row (Figure II-5), and each tooth has a width-to-length ratio of approximately 1:2. These teeth have a rake angle (the angle of the face of the tooth to the normal to the working surface; Figure II-6) near 50°, and a clearance angle (the angle from the working surface to the back of the tooth; Figure II-6) of 0° to 10°, depending on the amount of wear on the teeth. The total amount of tooth area in contact with an alga was 97 $\mu m^2/\mu m$ length of radula, or 1.2×10^6 $\mu m^2/5$ rows of teeth (the average number of rows in contact with the surface during a single motion of the radula). *Tectura scutum* has four effective teeth per row, with the first lateral teeth being longer than the second (Figure II-7). The third lateral teeth are greatly reduced and fused to the second lateral teeth. As the first lateral teeth are longer and in the same path as the second lateral teeth, they were used for the scale model. Compared to the teeth of *A. mitra*, they are proportionally longer and thinner, with a width-to-length ratio of 1:3 or 1:4, depending on the amount of wear. They have a rake angle of approximately 20° (it was impossible to measure this angle exactly from the profiles as these teeth are concave), and a clearance angle of about 50°. The surface area in contact with the substrate was 13 $\mu m^2/\mu m$ length of radula, or 1.6×10^5 $\mu m^2/5$ rows of teeth; one eighth of the tooth area in contact with the substratum of *A. mitra*.

Effect of tooth orientation

When the model teeth were scraped across plaster of Paris, the _Tectura scutum_ model was more effective at a wider range of tooth angles (-45° to $+45^{\circ}$) than the _Acmaea mitra_ model. The _A. mitra_ model was effective at the normal position (0°) and negative angles, but less effective at positive angles where friction caused by the low clearance angle resulted in the tooth sliding along the surface rather than cutting it.

When the radula of _Acmaea mitra_ was reversed to test the importance of tooth angles, the rake angle changed from $+50^{\circ}$ to -90° and the clearance angle from $+10^{\circ}$ to $+120^{\circ}$. The forces required to pull the algae past the radula were quite different than for the radula in the natural feeding position (Figure II-4).

DISCUSSION

Researchers have placed algae into groups based on thallus form and have claimed that these groups exhibit differential resistance to herbivores (Littler and Littler 1980; Steneck and Watling 1982). These models predict that the thin blade would be the least resistant and the crustose coralline would be the most resistant to herbivores. The results of the above experiments were the inverse of the predictions of these models. This discrepancy could be a result of the experimental methods that were used by Littler and Littler (1980) and Steneck and Watling (1982) to predict structural resistance. The experiments reported here used actual radulae of herbivorous gastropods and mimicked the manner in which these limpets use their radulae. Littler and Littler (1980) used a penetrometer (a plunger-like apparatus) and measured the force required to push this plunger through the algae, referring to this as "toughness". This plunger had rounded edges, a surface area four to five orders of magnitude greater than the area of the feeding apparatus of a limpet, and did not mimic the feeding behavior of any marine herbivore examined to date (fishes, crabs, sea urchins or molluscs; personal observation). Steneck and Watling (1982) determined the "scratchability" of algae using a stylette (of unmentioned material), referring to this as "toughness". Such scratch tests, however, are a measure of hardness, and do not take into account the importance of tooth composition and the consequences of different degrees of normal force applied, both of which were shown to be important in this study.

The ability of plants to resist tissue loss to herbivores depends on the properties of the algae as well as the feeding apparatus of the herbivore. The more energy required to remove tissue from a plant, the more difficult that plant will be to eat.

Materials may have different mechanical properties when forces are applied to them in different ways (i.e., a material may be more resistant to breakage in compression than in tension, or more resistant to forces applied in one direction than another; Wainwright et al. 1975). Thus, to determine a plant's ability to resist tissue loss to a particular herbivore, it is important to mimic the manner in which the herbivore feeds.

The hardness of a material is generally measured as the ability of one material to scratch another; hardness is poorly correlated with the amount of energy that is required to break that material. If the feeding apparatus of the herbivore is not as hard as the algal material, the herbivore will not be able to scratch the plant. However, limpets and many other herbivorous molluscs have radulae enriched with iron and silica (Lowenstam 1962, 1981; Runham et al. 1969), both of which are much harder than calcified algae. Although the teeth of limpets are capable of scratching all types of algal tissue, it still requires different amounts of force (and therefore energy) to remove tissue from different types of algae.

In the coralline algae, calcification occurs in the form of minute crystals of calcite, which form an integral part of the cell walls (there is no intercellular calcification; Borowitzka 1982). Coralline algal tissue, like many biological ceramics, is very brittle and breaks rather than bends under applied forces (Wainwright et al. 1975). The fleshy algae, on the other hand, are flexible, and initially stretch under applied forces, therefore requiring more energy to break them.

An alga that is very resistant to tissue loss against one consumer may not be resistant to another that feeds in a different manner or that has a feeding apparatus with different mechanical properties. For the consumer to be an effective feeder on a particular type of alga, its teeth must be minimally harder than the algal materials. In addition, the numbers of teeth and the surface area of the teeth in contact with the alga influence the tip stress of the teeth (force per unit area of tooth in contact). The fewer the number of teeth and the smaller the surface area in contact with the alga, the greater the applied force (and stress) at each tooth for a given force applied to the entire radula. For example, the total surface area of the teeth in contact with an alga is eight times greater for Acmaea mitra than it is for Tectura scutum. Therefore, for any force applied to the entire radula, the actual force at each tooth tip will be eight times greater for T. scutum than for A. mitra. This difference may explain why the normal force required to remove tissue from noncalcified algae was much greater for A. mitra than for T. scutum.

Acmaea mitra and Tectura scutum differ in the shapes of their teeth, particularly in the clearance angles. In machining, the rake and clearance angles are critical aspects of abrasive tools (Figure II-5). Mulhearn and Samules (1962) and Samules (1978) determined that there is some critical value for the rake angle of an abrasive tool to be effective at removing material; the critical angle is dependent on the material being abraded. If the tool is held at a rake angle more positive than the critical angle, it will cut the material (cleanly remove material); otherwise, the tool tends to plow (push material to the side). The actual magnitude of the rake angle, if it is greater than the critical angle, is unimportant until it becomes so positive that the clearance angle is decreased to the point where friction occurs between the cutting surface and the substratum (Samules 1978).

For soft tissues, such as bladed algae, a 0° clearance angle may result in the tooth sliding across the surface rather than cutting it (e.g., *Acmaea mitra* teeth on *Hedophyllum sessile* and *Iridaea cordata*). On a material that is harder, such as a calcified alga, a 0° clearance angle would result in rapid wear of the tooth, but this wear may in fact make the teeth more effective tools. Runham and Thornton (1967) found, when studying the the mechanical wear of of the mineralized teeth of the limpet *Patella vulgata*, that initially the tips of the teeth break off and then the teeth wear to an effective chisel shape. Runham et al. (1969) found that for *P. vulgata* there was differential wear on different portions of the tooth that maintained a sharpened edge on the tooth, essentially like a self-sharpening knife. This worn configuration could therefore be the most effective shape for these teeth (Hickman 1980).

Although the thallus form may be important in influencing the impact of herbivores (or other disturbance agents) on algae, it may act more through influencing availability of the plant or causing difficulty in handling, rather than by influencing structural resistance to tissue loss. In any case, the material properties of the alga, the properties of the feeding apparatus and mode of use of the feeding apparatus of the herbivores are important components in determining algal susceptibility to herbivores.

Both Littler and Littler (1980) and Steneck and Watling (1982) chose to describe physical resistance to herbivores as "toughness", although they were measuring different properties in different ways. Engineers generally use the term toughness to refer to the ability of a material to resist the propagation of critical cracks (cracks or breaks in the material that result in structural failure), but even this is not totally agreed on (Gordon 1968; Wainwright et al. 1975; Vincent 1982). Terms such as toughness carry both technical and colloquial meanings and connotations, and consequently result in much confusion. I therefore suggest that terms such as toughness should not be used unless carefully and explicitly defined.

Present functional group and functional form models do not appear to be good predictors of algal resistance to herbivores. Rather, one must consider other material properties of the plant, the feeding apparatus of the herbivore, and mode of feeding.

Figure II-1. Generalized longitudinal section through the anterior portion of a limpet, showing the position of the radula relative to the mouth and oesophagus (adapted from Barnes 1980 and Fretter and Graham, 1962).

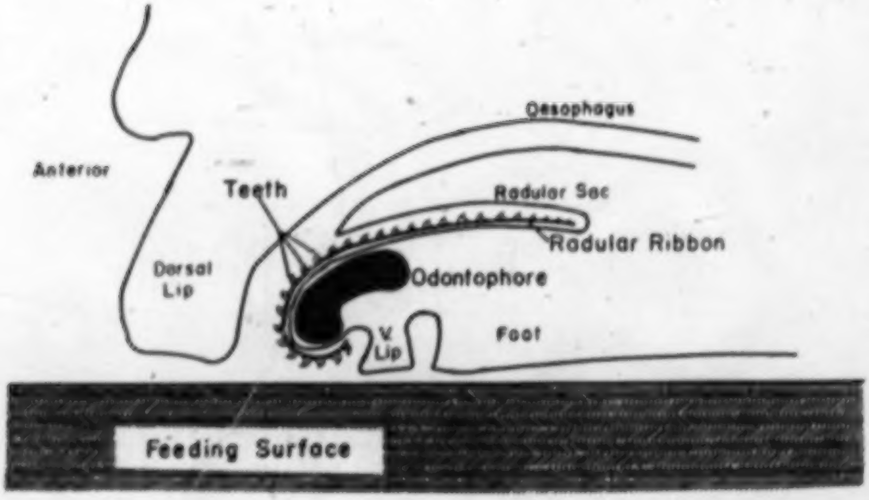

Oesophagus
Anterior
Teeth
Radular Sac
Radular Ribbon
Dorsal Lip
Odontophore
V. Lip
Foot
Feeding Surface

Figure II-2. (A) Drawing of a radula attached to the Plexiglas support, placing it parallel to the alga. (B) Apparatus used to determine the forces required to pull the alga past the radula. The normal force, lateral force, velocity and distance the radula traveled were simultaneously recorded.

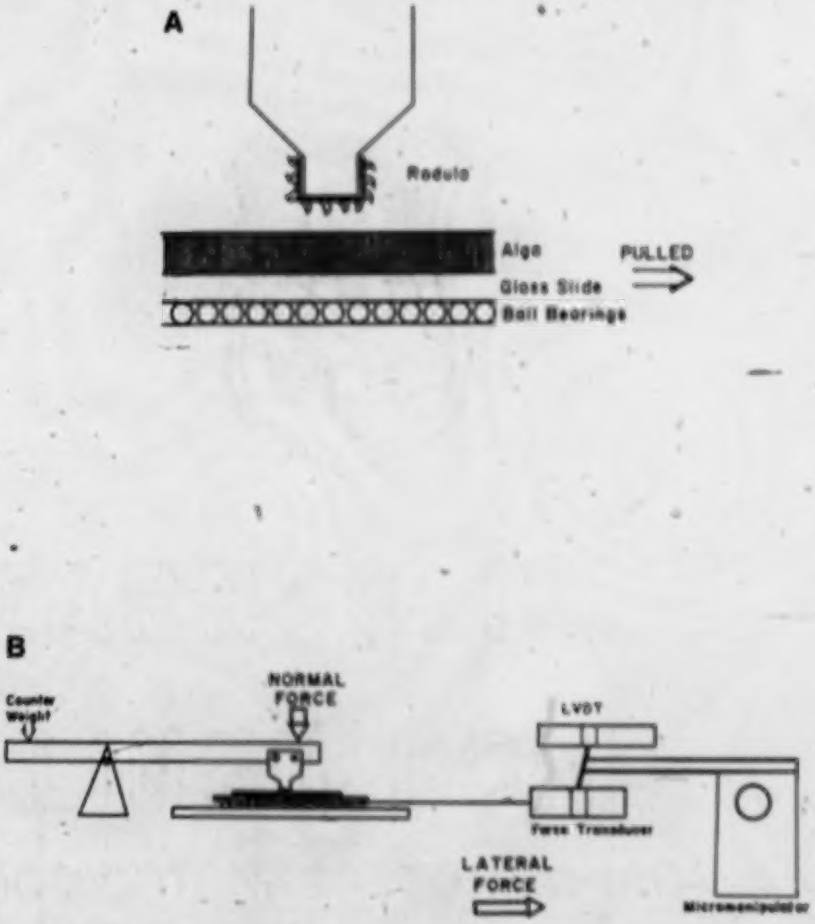

A
Radula
Alga
PULLED
Glass Slide
Ball Bearings
B
Counter Weight
NORMAL FORCE
LVDT
Force Transducer
LATERAL FORCE
Micromanipulator

Figure II-3. Minimum normal force required for *Acmaea mitra* and *Tectura scutum* to remove tissue from *Iridaea cordata*, *Hedophyllum sessile*, and *Pseudolfthophyllum whidbeyensi*. Although the absolute values were not always equal, the rank order of vulnerability of the algae for the two limpets was the same. (In all cases the variance was immeasurable, therefore these differences are significant).

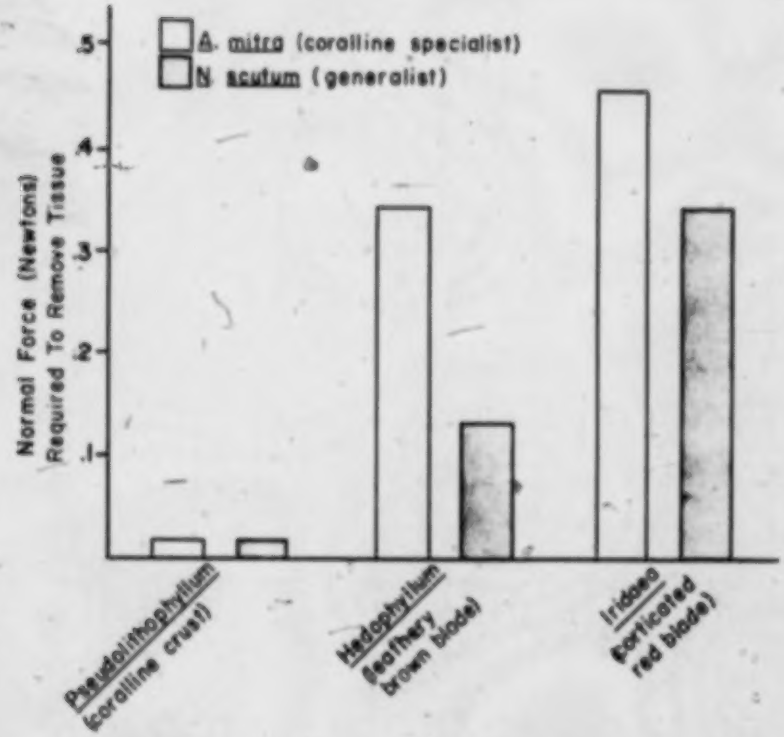


Figure II-4. Lateral forces required to pull algae from three genera (*Iridaea*, *Hedophyllum*, and *Pseudolithophyllum*) past the radulae of *Tectura scutum* and *Acmaea mitra* in normal and reversed feeding positions vs the normal force applied. The minimum force required to remove tissue is indicated (*). (Lines represent the least-squares regression of the data; r = correlation coefficient).

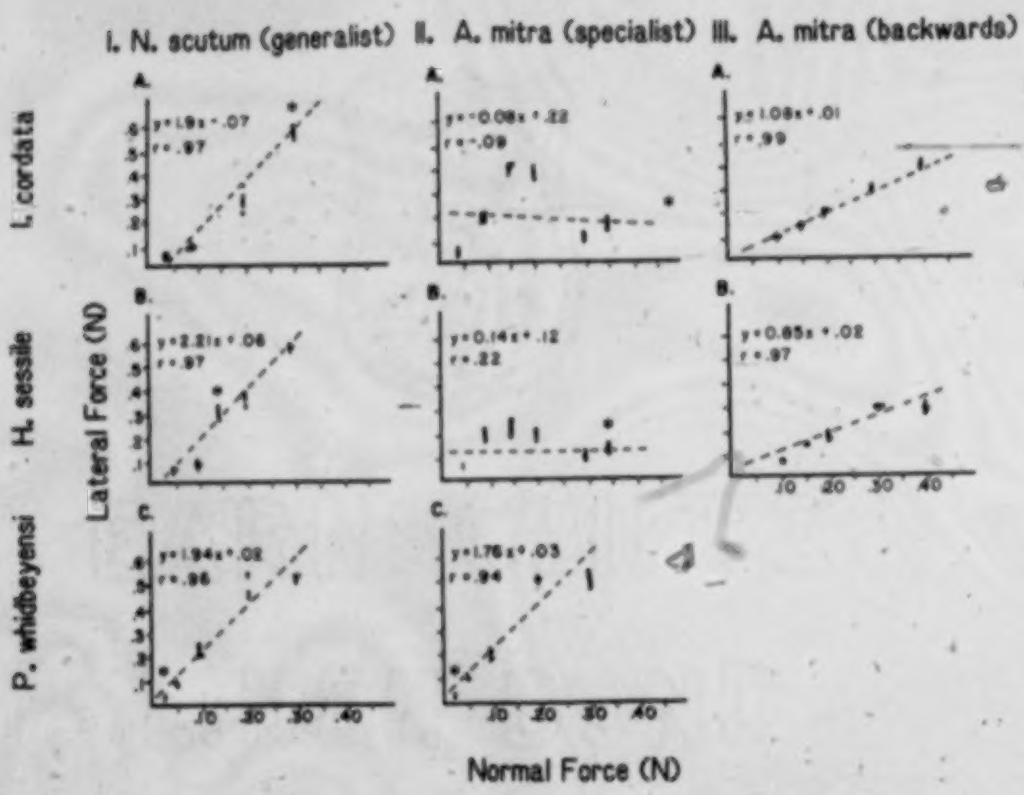


Figure II-5. Acmaea mitra. Side (A) and front (B) views of the radula of A. mitra. The scale bar = 100 µm.

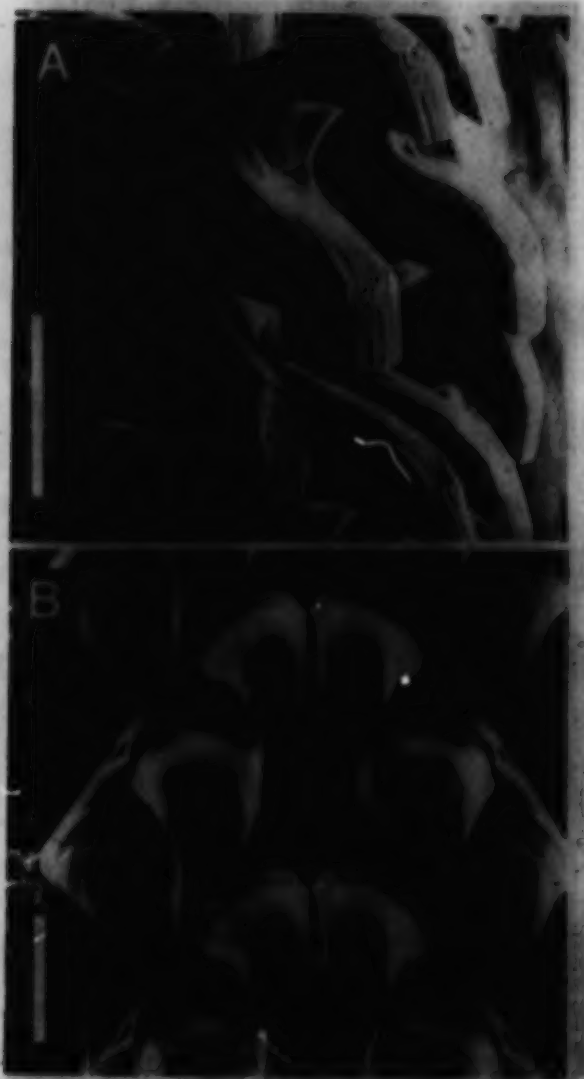


Figure II-6. Rake angle (measured from normal to the work piece) and clearance angle (measured from the surface of the work piece) for an abrasive tool. The corresponding angles of a radular tooth are also indicated.

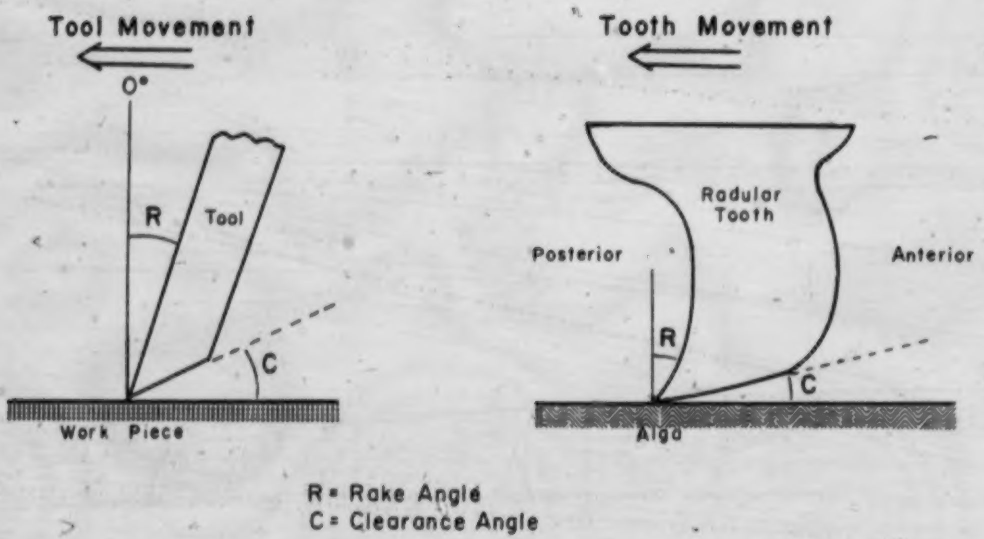
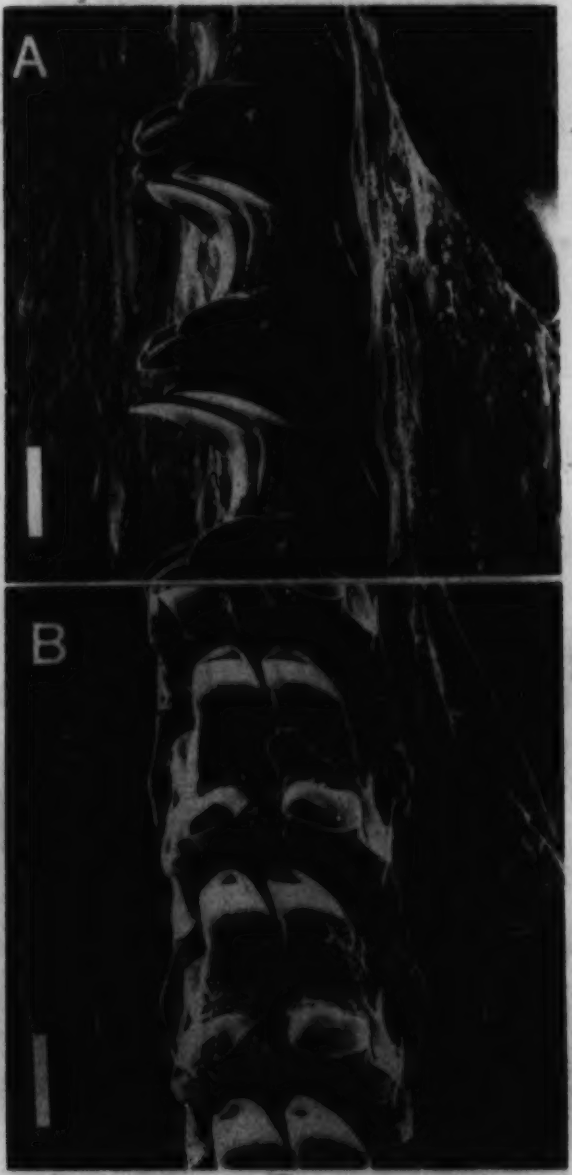

Tool Movement
0°
R
Tool
C
Work Piece
Tooth Movement
Radular Tooth
Posterior
Anterior
R
C
Alga
R = Rake Angle
C = Clearance Angle

Figure II-7. Tectura scutum. Side (A) and front (B) views of a worn radula of T. scutum. The teeth are more worn anteriorly and less worn posteriorly (anterior: top A and B).



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Chapter III. Mechanical resistance of algae against grazers: the importance of form and calcification in influencing resistance to tropical docoglossan limpets.

INTRODUCTION

Marine macrophytic algae occur in a variety of growth forms and many are calcified in various ways and to varying degrees (Borowitzka 1982). Several factors probably influenced the evolution of algal form and algal calcification, including light, wave motion, nutrient availability and herbivores (Littler and Littler 1980, Borowitzka 1982, Steneck and Watling 1982, Norton et al. 1981, 1982, Steneck 1986). Calcification is thought to have evolved primarily as a structural defense against herbivores (Johansen 1981, Steneck 1982, 1983), which increases the alga's mechanical resistance to tissue loss to herbivores. Littler and Littler (1980) and Littler et al. (1983) have constructed a model predicting many aspects of the ecology of marine macrophytes, including susceptibility to herbivores, on the basis of thallus form and degree of calcification. Steneck and Watling (1982), incorporating a consideration of the mode of feeding of the herbivore, have proposed a similar scheme predicting algal susceptibility to molluscan herbivores. Although the scheme of Steneck and Watling (1982) differs slightly from that of Littler and Littler (1980) for filamentous algae and non-calcified crustose algae, the overall patterns of algal susceptibility they predict are similar (Table III-1). Some correlational data support these models; however, many other confounding factors may influence plant-herbivore interactions including anti-herbivore chemicals, food preferences or nutritional considerations for the herbivore. Very few direct tests of these models are not confounded by other factors (but see Chapter II and Watson and Norton 1985).

The predictions of these functional form models and of the hypothesis that calcification acts primarily as a structural defense were tested for a wide variety of tropical macrophytes and several species of tropical limpets, using an experimental method that directly measures algal resistance to tissue loss by the radulae of docoglossan limpets. This method (Chapter II) allows one to measure the structural resistance of an alga to tissue loss by an herbivore without being confounded by the other factors mentioned above. It thus allows a direct test of the predictions of the structural defense models for a group of ecologically important grazers in many marine systems around the world, docoglossan limpets (Branch 1981). If algal calcification and thallus form have evolved primarily in response to the selection pressures of marine grazers, one would predict that the mechanical resistance of algae to this major group of grazers would increase with increasing degree of calcification and with different types of thallus forms (Table III-1).

MATERIALS AND METHODS

This research was conducted at the Smithsonian Tropical Research Institute marine laboratory at Punta Galeta (9°24′21″N, 79°52′18″W) on the Caribbean coast of the Republic of Panama. Limpets and algae were collected from shallow algal reefs, the Galeta Lab reef and similar reefs to the west at Isla Margarita, periodically exposed during times of low water. The floral and faunal diversity of these sites have been described elsewhere (Cubit and Williams 1983, Hay 1984).

Three Caribbean limpet species were used in experiments: Collisella tranquebarica, Tectura elegans, and T. albicosta. Species of reef algae varying in thallus form and degree of calcification from three algal divisions were examined: the green algae (Chlorophyta) Halimeda opuntia, H. tuna, H. discoida, Udotea sp., Caulerpa mexicanum, Dictyospharea sp., Anadyomene stellata; the brown algae (Phaeophyta) Dictyota bayteresia, Padina jamacensi, Sargassum natans; and the red algae (Rhodophyta) Amphiroa hancockii, Sporolithon sp., and Hydrolithon sp. (Table III-2).

Algal susceptibility to limpet rasping was determined by measuring the forces required to remove tissue. The radulae were carefully removed from freshly sacrificed animals, cleaned of all tissue with fine dissection needles, and kept moist until used. The anterior most portion of each radula (not including the first 3 to 4 rows of teeth) was attached with a cyanoacrylate adhesive to a Plexiglas support (2.2 mm wide), placing the radula in feeding position, with four to six rows of teeth on the working surface of the support. The support was attached to a beam placing the radular ribbon parallel to an alga placed beneath it. The beam was counterbalanced in such a way that the radula and support placed no force on the alga. Various loads (= normal forces) were applied to the radula by attaching weights with dual adhesive tape to the beam directly above the radula.

The alga being tested was attached at one end with a cyanoacrylate glue to a glass slide which rode on tracks of ball bearings, thus minimizing friction, and was attached to a force transducer mounted on a micromanipulator. Thus, when the alga was pulled past the radula with the micromanipulator, the force required to do so (= lateral force) could be determined.

A linearly variable differential transformer (LVDT, Pickering and Co., Inc. model 7307 W3-AO) was mounted on the micromanipulator to determine the velocity at which the alga was moved. The LVDT measures the distance the alga travels, and using the chart speed of the recorder as a time base one can calculate the velocity as the slope of the charted displacement curve. These velocities were matched to those of radulae in live limpets when feeding (1.0 to 1.5 cm/s).

Several radulae of each species of limpet were tested for each of the algal species. If the radula lost teeth, came loose or appeared very worn it was replaced. At least ten replicate trials each were made at each of a range of normal forces (0.0098 N to 0.4900 N) for each alga species-limpet species combination tested. Initially, normal forces were increased by coarse increments of 0.0490 or 0.0980 N until tissue was removed. Then the normal force applied was decreased by a finer increment of 0.0098 N to determine the minimum force to remove tissue. After each test, the alga was examined under a dissecting microscope (at 250x magnification) to determine if tissue had been removed. If it was difficult to discern if tissue had been removed, the surface of the alga was painted with a dilute solution of white, water soluble ink. This allowed one to focus on the opaque particles and determine if any tissue had been removed.

To test whether the models in Table III-1 were accurate predictors of algal mechanical resistance to these limpet herbivores, the minimum force required to remove tissue for each of the algal/limpet combinations was compared among groups. Due to unequal variance, a Kruskal-Wallis Test (Zar 1974) was used to determine if there was a significant differences among groups and then a Student-Newman-Keuls Test (Zar 1974) was used to determine which groups were different.

RESULTS

The relationship between the force required to remove tissue from algae and the degree of calcification and thallus form were consistent among all of the limpet species tested. The minimum normal force required to remove tissue from algae decreased with increasing amounts of calcification (Figure III-1). The algae were divided into three groups: noncalcified, lightly calcified, and heavily calcified. The mean minimum normal force required to remove tissue for each of the three groups differed significantly (Kruskal-Wallis Test, $p<0.01$; Student-Newman-Keuls Test, $p<0.05$; Zar 1974). The most heavily calcified algae required the least amount of force to remove tissue while the noncalcified plants required the most (Figure III-1). The means of the normal forces required to remove tissue from the crustose, jointed calcareous, and leathery plants were less than those for the sheets and coarsely branched plants (Kruskal-Wallis Test, $p<0.01$; Student-Newman-Keuls Test, $p<0.05$; Figure III-2). In all cases the variance in the minimal normal force required to remove tissue was not detectable for each limpet-algal species combination. This was probably due to the force increments (0.0098 N) being large relative to the intraspecific variance in resistance. The minimum normal force and subsequent lateral force required for each alga-limpet pair tested are in Table III-3.

DISCUSSION

Structural defenses for plants are an important first line of defense against herbivores. The structural resistance of a plant against herbivores will influence the selective advantage that chemical or nutritional defenses impart, because these latter defenses generally require that the herbivore taste the plant. If an herbivore is unable to remove tissue from a plant because of its structural properties, chemical and nutritional qualities will be of little consequence. Structural defenses in general and calcification in particular are very important to consider regarding the evolution of anti-herbivore defenses because, unlike chemical properties or behavioral responses of herbivores, they may be traced in the fossil record (Wray 1975, Flugel 1977).

The experiments discussed above were conducted to determine if thallus form and calcification could act as structural defenses against grazing by docoglossan limpets. The results are, in general, contrary to predictions made by the three hypotheses regarding the importance of algal form and algal calcification in influencing algal resistance to herbivores. But, they are consistent with previous work on temperate zone algae and two limpet species (Chapter II). In all cases, increasing amounts of calcification decreased the plant's resistance to tissue loss. Non-calcified plants required more force for tissue to be removed than did calcified plants. This is probably because calcified plants are brittle and tend to break when forces are applied. The fleshy algae, on the other hand, yield under applied force, absorbing energy, and require greater total force to break (Chapter II).

The functional groups of Littler and Littler (1980) and Steneck and Watling (1982) were very poor predictors of mechanical resistance to herbivores as well. In general, the patterns of mechanical resistance as a function of thallus form were contrary to the predictions of both the Littler and Littler and Steneck and Watling models.

Obviously structure is not the only factor influencing algal susceptibility to herbivores. However, understanding the potential for calcification to act as a structural barrier against herbivores may shed light on previously assumed dilemmas about the role of chemical defenses in heavily calcified plants (Paul and Fenical 1983, Paul and Hay 1986). Heavily calcified plants may in fact not be well defended mechanically against all forms of grazers and may therefore rely on chemical defensive compounds. Also, the degree to which thallus form and calcification influence algal susceptibility to docoglossan limpets may not be the same for other herbivores which feed in a different manner or have a different feeding apparatus (Chapters IV and V). It is important to separate the mechanical resistance of algae to herbivores from feeding preferences when assessing algal defenses, and the consequences of defenses for both the plants and the herbivores.

Table III-1. Predictions of three models regarding the structural resistance of macroalgae to herbivores.

FUNCTIONAL FORM (1)	FUNCTIONAL GROUP (2)	CALCIFICATION (3)	SUSCEPTIBILITY
SHEET	FILAMENTOUS	NON-CALCIFIED	MOST SUSCEPTIBLE
FILAMENTOUS	FOLIOSE		
COARSELY BRANCHED	CORTICATED		
THICK LEATHERY	LEATHERY	LIGHTLY CALCIFIED	
JOINTED CALCAREOUS	ARTICULATED CALCAREOUS		
CRUSTOSE	CRUSTOSE CORALLINE	HEAVILY CALCIFIED	LEAST SUSCEPTIBLE

(1) Littler and Littler 1980
(2) Steneck and Watling 1982
(3) Johansen 1981

Table III-2. Algae that were used in this study and the calcification and form groups to which they were assigned (Littler, Littler and Taylor 1983). Algae are arranged according to Division.

ALGA	FUNCTIONAL GROUP	CALCIFICATION
Chlorophyta:		
ANADYOMENE	SHEET	NONE
CAULERPA MEXICANUM	COARSELY BRANCHED	NONE
DICTYOSPHAEREA	SHEET	LIGHT
HALIMEDA DISCOIDA	JOINTED CALCAREOUS	HEAVY
HALIMEDA OPUNTIA	JOINTED CALCAREOUS	HEAVY
HALIMEDA TUNA	JOINTED CALCAREOUS	HEAVY
UDOTEA	LEATHERY	LIGHT
Phaeophyta:		
DICTYOTA	SHEET	NONE
PADINA	LEATHERY	HEAVY
SARGASSUM	LEATHERY	NONE
Rhodophyta:		
AMPHIROA HANCOCKII	JOINTED CALCAREOUS	HEAVY
HYDROLITHON	CALCIFIED	HEAVY
NEOGONOLITHON	CALCIFIED	HEAVY
SPOROLITHON	CALCIFIED	HEAVY

Table III-3. Minimum normal force measured and resultant lateral force to remove tissue from each algal species by each limpet species (TE= *Tectura elegans*, TA= *T. albicosta*, and CT= *Collisella tranquebarica*). Algae are arranged according to division. * indicates that this was the lowest force tested.

ALGA	LIMPET	NORMAL FORCE (N)	LATERAL FORCE[a] (N)
Chlorophyta:			
ANADYOMENE	TE	.1470	.10140 (.00131)
	TA	.1470	.05146 (.00951)
	CT	.1470	.07941 (.00310)
CAULERPA MEXICANUM	TE	.1470	.05938 (.00103)
	TA	.0980	.04850 (.00088)
DICTYOSPHAEREA	TE	.0980	.03027 (.00065)
HALIMEDA DISCOIDA	TE	.0294	.03272 (.00083)
HALIMEDA OPUNTIA	TE	.0098*	.01473 (.00021)
HALIMEDA TUNA	TA	.0294	.02508 (.00063)
UDOTEA	TE	.0294	.02384 (.00042)
Phaeophyta:			
DICTYOTA	TE	.0294	.01915 (.00019)
	TA	.0686	.03089 (.00084)
PADINA	TA	.0098*	.00905 (.00016)
	CT	.0098*	.01743 (.00238)
SARGASSUM	TE	.1470	.04470 (.00156)
Rhodophyta:			
AMPHIROA HANCOCKII	TA	.0098*	.00585 (.00014)
	TE	.0098*	.00275 (.00056)
	CT	.0098*	.00254 (.00023)
HYDROLITHON	CT	.0098*	.00800 (.00062)
NEOGONOLITHON	TE	.0294*	.01819 (.00062)
SPOROLITHON	TE	.0098*	.00379 (.00010)

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[a] Table entries are the means with standard errors in parentheses.

Figure III-1. Minimum normal force required for three limpet species (C. tranquebarica, T. elegans, and T. albicosta) to remove tissue from algae as a function of degree of calcification. Algae are classified according to degree of calcification into three groups: noncalcified, lightly calcified, and heavily calcified. Bars represent the average minimum normal forcce with sample sizes above. The means of these three groups are significantly different (Student-Newman-Keuls Test, p<0.05). For each limpet species/algal species combination the variance was not measurable.

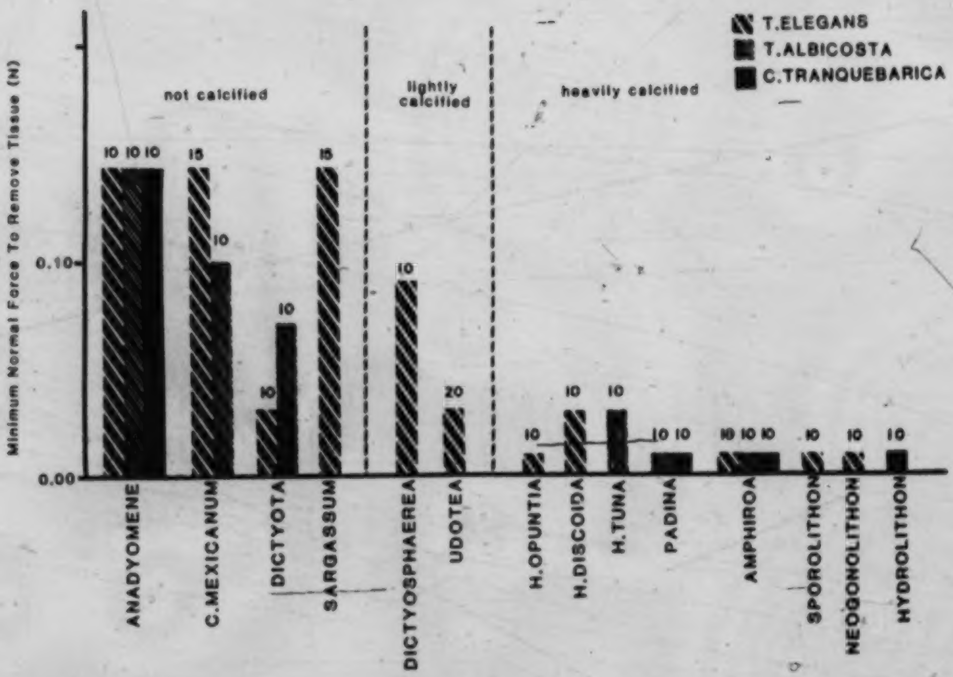
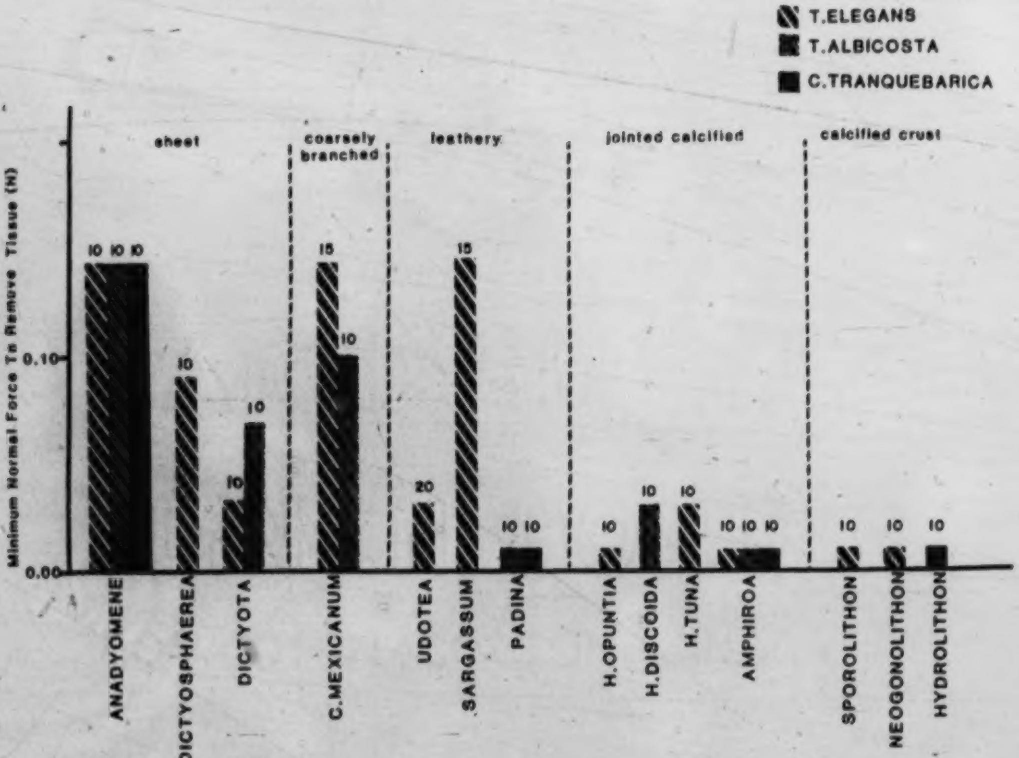


Figure III-2. Minimum normal force required for three limpet species (*C. tranquebarica*, *T. elegans*, and *T. albicosta*) to remove tissue from algae as a function of algal Functional Groups (Littler and Littler 1980, Steneck and Watling 1982). Bars represent the average minimum normal forcce with sample sizes above. The means for the sheet-group and the coarsely branched group differ significantly from those for the leathery-group, jointed calcareous group, and calcareous crust-group (Student-Newman-Keuls Test, $p<0.05$). In all cases the variance was not measurable.



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Chapter IV. The importance of mode of feeding in algal structural defenses: Algal resistance to an herbivorous crab.

INTRODUCTION

Marine macrophytes occur in abundance in a wide variety of types and forms throughout the world. They are exposed to a variety of herbivores which feed in very different ways, including molluscs, sea urchins, crustaceans, and fishes. Herbivores influence the distribution and abundance of macrophytes in many systems (see reviews by Lubchenco and Gaines 1981, Gaines and Lubchenco 1982, Hawkins and Hartnoll 1983). Recently, researchers have recognized the importance of different types of herbivores in influencing community structure (e.g., Menge et al. 1986). The herbivores in a complex system can vary quantitatively in their effects on and importance to plants, and more importantly they can differ qualitatively. As plants in complex systems are usually confronted with more than one type of herbivore the impact of each of the different types of herbivores must be considered, regarding both the ecology and the evolution of antiherbivore traits. However, it is often difficult to determine what attributes of algae influence their vulnerability or desirability to herbivores.

When considering structural defenses of plants against their herbivores several different factors are important:

the mode of feeding of the herbivore, the material and structural properties of the feeding apparatus, and the material and structural properties of the plant at the scale appropriate to the particular herbivore in question. When considering the evolution of plant defenses one must consider the possibility that the present herbivores may not have been the most important herbivores during the evolution of the plant, and/or current herbivore preferences may not reflect evolutionary patterns in these defenses. Herbivores may have been important in influencing structural defenses of plants, but present nutritional responses or other factors may have an overriding influence on food choice.

One way to test whether herbivores could have been important in the evolution of plant form and structure is to determine the potential for structural defenses to act by mechanically mimicking the mode of feeding of the herbivores and measuring the mechanical resistance of the plant to tissue loss. This allows one to separate mechanical resistance to tissue loss from chemical defenses and nutritional and behavioral responses of the herbivore. An approach of this type can lead to very different results than would be obtained by more simple methods of treating all herbivores together or relying on herbivore preferences for suggestion of plant defenses.

The resistance of algae to the feeding mechanisms of one group of herbivores, the docoglossan limpets, has been examined in this way (Chapters II and III). Herbivorous crabs are another potentially important group of herbivores; they feed in a very different way from limpets and little is known about the resistance of algae to grazing by crabs. In tropical systems crabs are abundant, can attain large sizes, and have the potential to be important herbivores. Many of the large species remove tissue from algae by grasping the plant with the tips of one chela and pulling it towards their body until plant tissue is removed. This is then repeated with the other chela. The chela is not twisted or rotated (pers. obs.). The pieces of plant are taken to appendages at the mouth where they are manipulated and placed in the mouth (Warner 1977 and pers. obs). The type of stress and subsequent damage imposed on the plant by this herbivore is very similar to the way herbivorous fish feed. Therefore, both of these groups could be considered analogous "biting-type" herbivores and considered together (Hawkins and Hartnoll 1983).

The herbivorous crab, Mithrax spinosissimus, can attain very large size and be quite abundant (Hazlett and Ritschoff 1975). It is common throughout the Caribbean to Venezuela, and is a generalist herbivore. It consumes large quantities of algae daily (Chapter V) with large, spoon-shaped chelae, common to many herbivorous crabs (Warner 1977). The structural resistance of a wide variety of tropical algal species to herbivory by this crab was measured by using the actual chelae of the crab and mimicking the way in which the crabs use them to remove algal tissue. In this way the forces required for Mithrax to remove tissue from particular algae could be measured without the confounding influence of food preference. These data could then be used to test hypotheses that predict algal resistance to herbivores on the basis of structural features such as thallus form and degree of calcification (Littler and Littler 1980, Johansen 1981, Littler et al. 1983).

The functional form model of Littler and Littler (1980) places algae into functional groups including convergent anatomical, physiological and ecological features. This model suggests that, among other factors, herbivores have been very important in the evolution of plant form and structure and therefore predicts algal resistance to herbivores on the basis of plant form (Table IV-1). Plant calcification in general has also been assumed to act primarily as a structural defense, inhibiting tissue removal by herbivores (Johansen 1981; Steneck 1982, 1983; Hawkins and Hartnoll 1983). If this were the case one would predict increased algal resistance to herbivores with increasing amounts of calcification (Table IV-1).

MATERIALS AND METHODS

Experiments were conducted at the Smithsonian Tropical Research Institute marine laboratory at Punta Galeta on the Caribbean coast of the Republic of Panama. Algae were collected from shallow, algal reefs, the Galeta Lab reef and similar reefs to the west at Isla Margarita (Chapter III).

The apparatus for measuring the forces required to remove tissue from the algae for this herbivore was modified from that described in Chapter II. The chela of the crab was removed and cleaned of tissue and refrigerated or frozen in sea water until used. The chela was held with the propus attached to a plexiglas support with a cyanoacrylate adhesive. The dactylus was attached to a beam that was counterbalanced such that the chela just failed to close so that the chela alone was not applying a force on the alga. Different amounts of force (=normal force) could be applied directly above this attachment, causing the chela to close on an algal thallus placed between its tips. The other end of the alga was attached with a cyanoacrylate adhesive to a glass slide that rode on tracks of ball bearings, minimizing friction with the surface. The slide was attached to a force transducer mounted on a micromanipulator. A linearly variable differential transformer (LVDT, Pickering and Co., Inc. model 7307 W3-AO) which measured the distance the alga traveled was also mounted on the micromanipulator (Figure IV-1). Therefore, for a given amount of normal force the lateral force and distance the alga moved were simultaneously measured and recorded with a chart recorder. Using the chart speed of the chart recorder, one could calculate the velocity the alga was pulled to insure that replicates were performed at approximately the same velocity (0.9-1.5 cm/s).

Five different chelae were used (two from immature males and three from adult females, 115-125mm in carapace length). Each algal species was tested a minimum of 10 times, and as many as 40 at a range of normal forces (from 0.2940 to 1.4700 N). Single thalli were tested only once with a given normal force and then discarded. If thallus form was amenable, different parts of the same plant were tested to determine intra-plant variability to compare with the inter-plant variability in resistance to tissue loss. Tissue loss from the plant was determined for each trial.

Two Mithrax were collected at the Galeta Reef, and the rest were purchased live at local markets after they had been collected by fishermen on nearby reefs. The crab chelae used in this study were from animals that had recently died on the Galeta reef or were accidentally killed during other feeding experiments. The crabs from the Galeta reef were found early in the morning on days of extreme exposures. These animals were probably feeding on the central portion of the reef and were trapped when the water fell. They appear to be intolerant of aerial exposure and rarely venture out of water (Hazlett and Ritschoff 1975 and pers. obs).

Algae were collected fresh (within 12 hours) for each experiment. The algal species used were: the green algae (Chlorophyta) Anadyomene stellata, Caulerpa racemosa, C. sertulariodes, Cladophora sp., Halimeda opuntia, Udotea sp.; the red algae (Rhodophyta) Acanthophora spicifera, Amphiroa hancockii, Galaxaura oblongata, Laurencia papillosa; and the brown algae (Phaeophyta) Dictyota bayteresii, Padina jamaicensi, and Sargassum natans. The sea grass Thallasia testudinum was also common on the reefs and was used for comparison. The predicted order of susceptibility for each of these algal species was determined for each model on the basis of thallus shape and degree of calcification (Table IV-2).

RESULTS

The variability among replicates within the same plant and for each chela were equal to or greater than the variability among plants and chelae, so all data were pooled for each algal species at each normal force (Table IV-3). There was a large variability in the probability that a particular normal force would remove tissue from each of the algal species. To make comparisons, the percentage of the trials that were successful at removing tissue either by biting or tearing when three different representative normal forces, 0.49N, 0.98N, and 1.47N, had been applied is presented for each algal species (Table IV-4). The values for 0.98N normal force for each algal species were grouped and these groups were ordered according to the predicted susceptibilities for each of the two models predicting algal structural resistance to herbivores (Figures IV-2 and IV-3). These data were used as all algal species had been tested at that force. In both cases, there was no pattern in proportion of successful trials and the calcification groupings or the functional form groupings. There was no difference in the means among any of the groupings for either model (Kruskal-Wallis Test 0.95>p>0.90; Zar 1974).

Although it was a very poor estimator for most algal species due to the high variances, the least squares line was used to estimate the force required for tissue removal in 50% of the trials in an attempt to provide a means of comparing the algae (Table IV-5). If there was only one non-zero value, a straight line connecting the last zero value with the non-zero value was used to predict the force required to remove tissue in 50% of the trials. These values were compared when ranked according to degree of calcification and the functional groups of Littler and Littler (1980) to determine the relative susceptibilities of these plants to Mithrax (Figures IV-4 and IV-5). Again, no pattern emerged. There was no significant difference among the means of the different groupings for either of the models (Kruskal-Wallis Test, 0.95>p>0.90; Zar 1974).

DISCUSSION

The results of these experiments examining the potential mechanical resistance of a variety of marine macrophytes against tissue loss to M. spinosissimus, a biting type herbivore, differed from the predictions of the models which predicted algal structural resistance to herbivores on the basis of thallus shape or degree of calcification. Clearly these potential structural defenses were not acting in the ways predicted by these models. There was no trend in the amount of force required to remove tissue as a function of degree of calcification or thallus form.

Also, these results were different from those obtained for the mechanical resistance of similar algal species to docoglossan limpets (Chapters II and III). For the limpets there was no measurable variability in the minimum amount of normal force required to remove tissue from each algal species for each limpet species. This was not the case for the crab where the variability in the probability that a particular normal force would remove tissue both within and between individual plants was high. This was probably due to a higher variance in the structural integrity of the plants at the scale at which claws interact with algal tissue as compared to the radulae of limpets. The grazing of limpets affects the algae at the scale of the material it is made of rather than the entire structure of the thallus. We might predict more homogeneity in the mechanical properties of algal tissue than for the structure of the entire plant, which was the scale that was important for a large herbivorous crab. Factors that could influence this would include the past history of the plant including any previous damage due to herbivores or other sources.

These results with a crab differ qualitatively as well as quantitatively from those obtained with limpets. For the limpets the trends were opposite to those predicted by the models (Chapters II and III) while for the crabs there were no significant trends. This underscores the importance of differences in mode of feeding of the herbivores for the effectiveness of the potential structural defenses. A structural defense against one type of herbivore may in fact make a plant more vulnerable to another type of herbivore. Also, knowing the resistance of plants to one type of herbivore does not necessarily allow one to predict the plant's resistance to another herbivore feeding in a different way. Consequently, the ecological importance and potential evolution of structural defenses will depend on all of these factors simultaneously.

Current models which predict algal resistance to herbivores on the basis of plant form and calcification do not explain the patterns of mechanical resistance of macrophytes against two major groups of herbivores, the docoglossan limpets and herbivorous crabs. The results of these experiments indicate that neither thallus form or calcification are acting in a simple fashion as structural defenses against these types of herbivores. The structural resistance of plants against their herbivores will depend on the way the forces are applied, the material properties of the feeding apparatus, and plant properties appropriate to the scale of the damage imposed by the feeding apparatus of the herbivore. Because of the great differences in the suites of herbivores to which most plants will be exposed, it may not be possible to make general rules or predictions about the ecology and evolution of potential structural defenses of plants against their herbivores.

Table IV-1. Predictions of two models regarding the structural resistance of macroalgae to herbivores.

FUNCTIONAL FORM (1)	CALCIFICATION (2)	SUSCEPTIBILITY
SHEET	NON-CALCIFIED	MOST SUSCEPTIBLE
FILAMENTOUS		
COARSELY BRANCHED		
THICK LEATHERY	LIGHTLY CALCIFIED	
JOINTED CALCAREOUS		
CRUSTOSE	HEAVILY CALCIFIED	LEAST SUSCEPTIBLE

(1) Littler and Littler 1980
(2) Johansen 1981

Table IV-2. Algae that were used in this study and the calcification and form groups to which they were assigned. Algae are arranged according to Division.

ALGA	FUNCTIONAL GROUP	CALCIFICATION
Chlorophyta:		
ANADYOMENE	SHEET	NONE
CAULERPA RACEMOSA	COARSELY BRANCHED	NONE
CAULERPA SERTULARIODES	COARSELY BRANCHED	NONE
CLADOPHORA	FILAMENTOUS	NONE
CODIUM	COARSELY BRANCHED	NONE
HALIMEDA	JOINTED CALCAREOUS	HEAVY
UDOTEA	LEATHERY	LIGHT
Phaeophyta:		
DICTYOTA	SHEET	NONE
PADINA	LEATHERY	HEAVY
SARGASSUM	LEATHERY	NONE
Rhodophyta:		
ACANTHOPHORA	COARSELY BRANCHED	NONE
AMPHIROA HANCOCKII	JOINTED CALCAREOUS	HEAVY
GALAXAURA	JOINTED CALCAREOUS	HEAVY
LAURENCIA	COARSELY BRANCHED	NONE

Table IV-3. Examples of the variation among and within crab chelae and algal species in the probability of tissue removal and forces required.

A. For Dictyota with 0.49N normal force:
Table entries are the proportion of successful trials and the total force (N) required for each successful trial.

	Crab		
Plant	1	2	3
1	1/3 0.9934		
2	0/5	0/4	
3		1/5 0.0480	0/4
4	0/4	2/4 0.3202 0.0091	
Total=4/29	1/12	3/13	0/4

B. For Acanthophora with 1.47N normal force:
In all cases there was 100% tissue removal. Table entries are the total forces (N) required for each individual trial.

	Crab		
Plant	1	3	5
1	1.111 0.449 0.649 0.737		0.986 0.868 0.286 0.713
2		1.011 1.072 0.529 0.681 0.263 0.373 0.655	1.249 1.198 0.296

Table IV-4. Percent of trials in which tissue was removed when 0.49N, 0.98N, and 1.47N normal force were applied. Sample sizes are in parentheses. Question marks indicate there are no data. Algae are arranged according divisions within a noncalcified and a calcified group.

	Normal Force Applied (N)					
Alga	0.49		0.98		1.49	
Noncalcified:						
ANADYOMENE	?		26.3	(19)	21.4	(14)
CAULERPA RACEMOSA	0	(9)	0	(9)	0	(5)
CAULERPA SERTULARIODES	42.9	(14)	57.1	(7)	?	
CLADOPHORA	100	(59)	100*		100*	
CODIUM	0	(5)	0	(20)	0	(4)
DICTYOTA	10.2	(59)	100	(14)	100*	
SARGASSUM	0	(5)	0	(20)	0	(5)
ACANTHOPHORA	?		62.5	(8)	100	(32)
LAURENCIA	0	(16)	0	(12)	0	(5)
Calcified:						
HALIMEDA	0	(4)	100	(14)	100*	
UDOTEA	0	(5)	0	(5)	0	(4)
PADINA	0	(11)	41.2	(17)	?	
AMPHIROA	0	(4)	0	(12)	0	(5)
GALAXAURA	32.5	(40)	31.4	(35)	?	

* It was assumed that all forces greater than that which resulted in 100% successful trials also were 100% successful.

Table IV-5. Correlation coefficients of least-squares lines used to estimate the normal force required to remove tissue for those species in which there were more than one non-zero value to use. If there was only one non-zero value, the line used connected the last zero with the non-zero value.

Alga	Correlation Coefficient	Sample Size
Acanthophora	0.75	40
Anadyomene	0.46	33
C. sertulariodes	0.92	53
Dictyota	0.89	96
Galaxaura	0.94	75

Figure IV-1. The apparatus for determining the amount of force required to remove tissue from algae for the crab Mithrax spinosissimus.

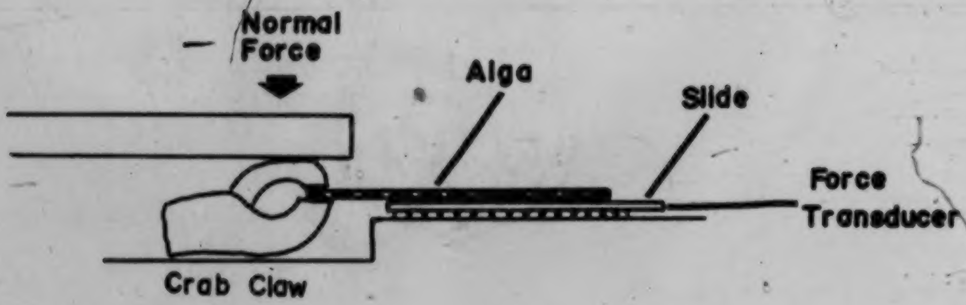

Normal Force
Alga
Slide
Force Transducer
Crab Claw

Figure IV-2. Percent of trials resulting in tissue loss with 0.98N normal force applied. Algae are grouped according to degree of calcification. Within each group alage are arranged according to division. Sample sizes are in parentheses.

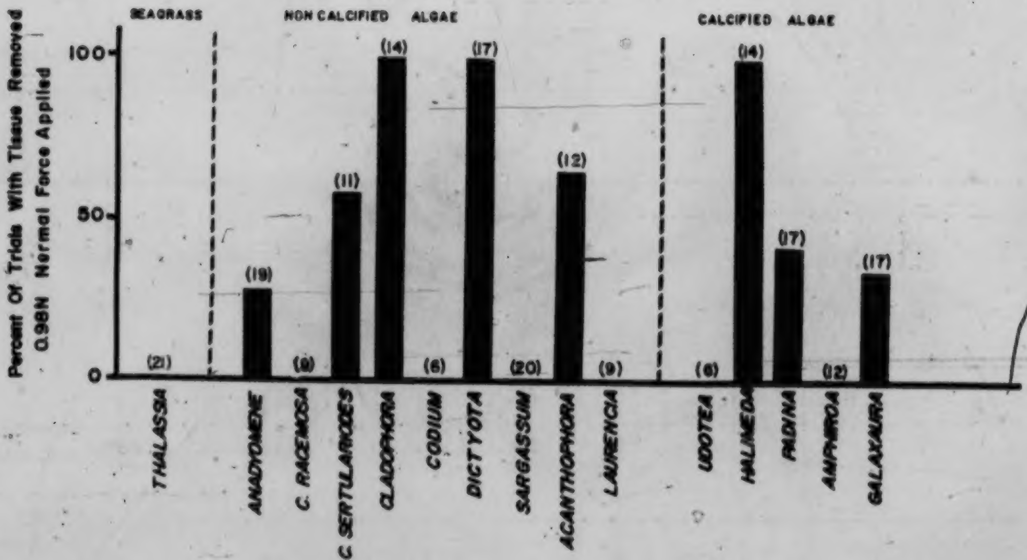


Figure IV-3. Percent of trials resulting in tissue loss with 0.98N normal force applied. Algae are grouped according to functional groups. Within each group algae are arranged according to division. Sample sizes are in parentheses.

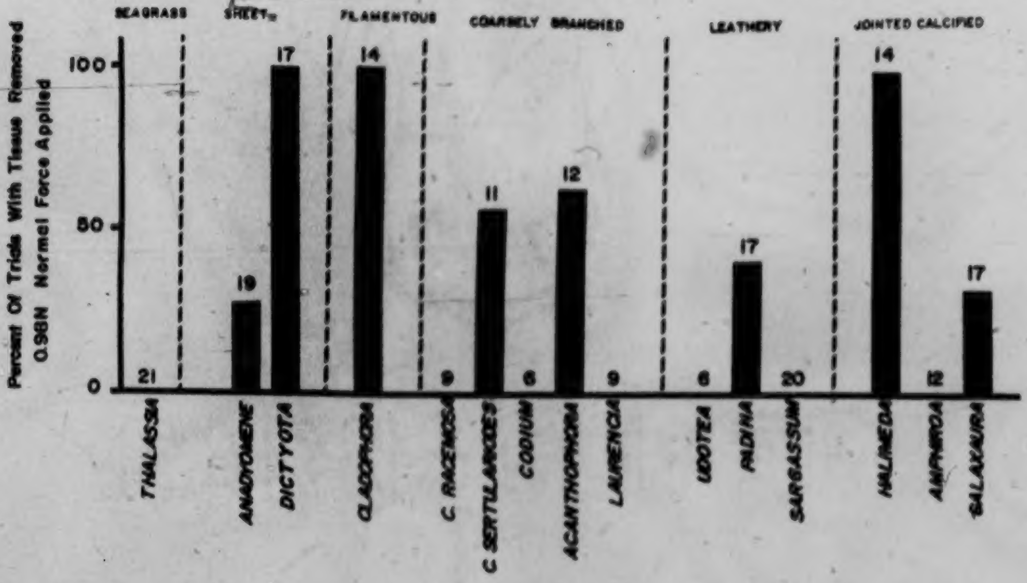


Figure IV-4. Estimates of the normal force required to remove tissue 50% of the time are plotted for each of the algae. Algae are grouped according to calcification. Within each group algae are arranged according to division. Open bars indicate that the estimate would be above the scale, but is unknown. Sample sizes are above bars.

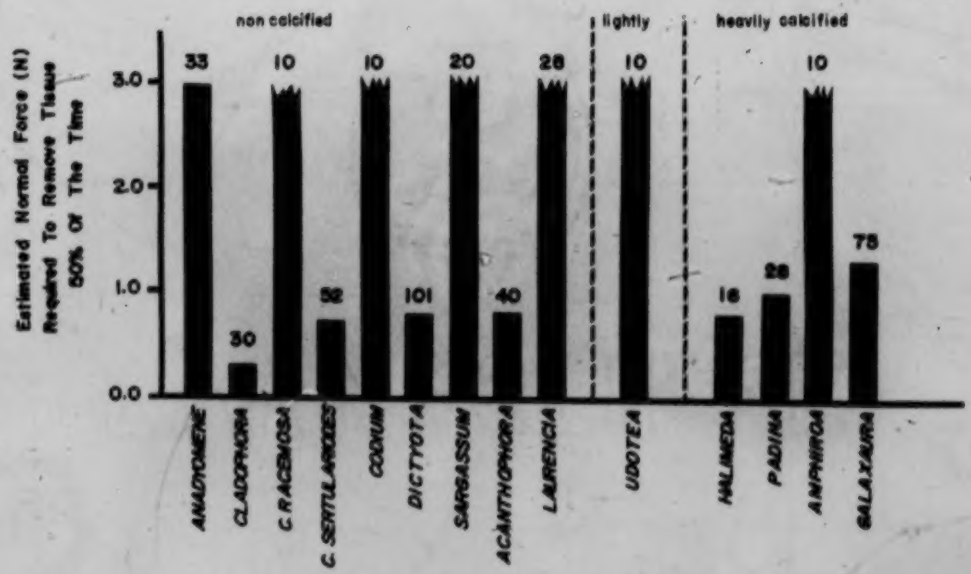
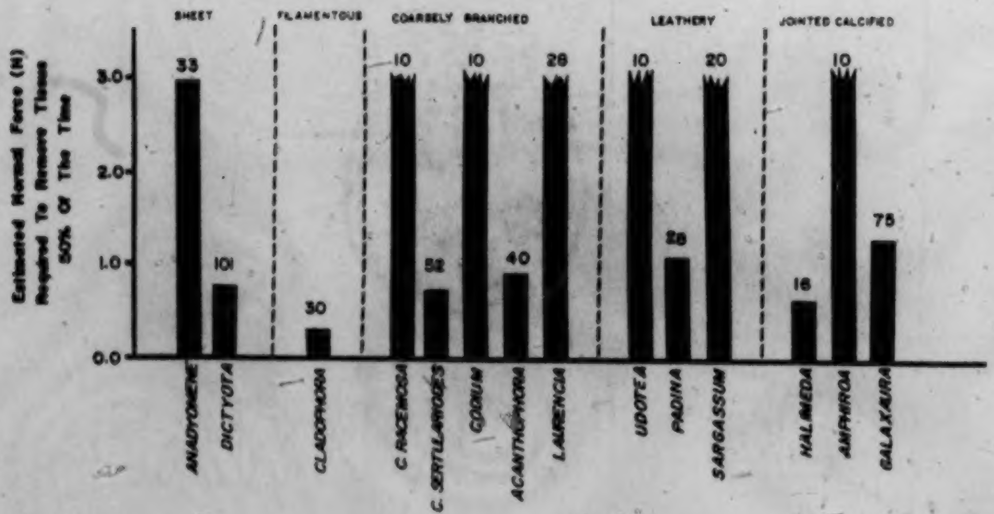


Figure IV-5. Estimates of the normal force required to remove tissue 50% of the time are plotted for each of the algae. Algae are grouped according to functional form groups. Within each group algae are arranged according to division. Open bars indicate that the estimate would be above the scale, but is unknown. Sample sizes are above bars.



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Chapter V. Feeding preferences of the herbivorous Caribbean spider crab Mithrax spinosissimus: relation to antiherbivore defenses.

INTRODUCTION

Mithrax spinosissimus (Lamarck) (Majidae, Decapoda), the West Indian Spider Crab or Spiny Spider Crab, occurs in the tropical western Atlantic from the Carolinas and Florida to the West Indies and Barbados, and throughout the Caribbean to Venezuela. It is the largest species of Mithrax in the region, and is found from shallow water to depths of 170 m along reefs or canals, feeding on macroalgae at night, and hiding in crevices or caves in the day (Hazlett and Ritschoff 1975). The claws of M. spinosissimus are massive and much larger in the males. The tips of the chelae are spoon-shaped, a common characteristic of herbivorous crabs (Williams 1984). M. spinosissimus is currently sold in local markets in parts of the Caribbean and is the target of mariculture projects because it spawns frequently, has a short larval phase (4 to 6 days), large size (males - 170 mm, females - 140 mm) and rapid growth (Provensano and Brownell 1977).

Very little is known about the feeding ecology of herbivorous crabs, although there is a growing literature about potential algal defenses against marine herbivores (both structural and chemical; Norris and Fenical 1982, Littler et al. 1983, Paul and Hay 1986). However, very few experimental tests have been made of the importance of potential defenses with the natural herbivores of the algae.

The purpose of this study was to determine the feeding preferences of M. spinosissimus when given a choice of six common reef algae. Actual food preferences could then be compared with preferences predicted when considering potential algal defenses to determine if these play a role in influencing food choice by this crab.

MATERIALS AND METHODS

Experiments were conducted at the Galeta marine laboratory of the Smithsonian Tropical Research Institute on the Caribbean coast of the Republic of Panama (Chapter II).

Adult Crab Experiments

Animals were measured, weighed and isolated in outdoor, flow-through running sea water tanks approximately 2m x 60cm x 30cm deep. The tanks were partitioned with plastic mesh (1 cm mesh) such that each animal was contained within an area of approximately 60 cm by 60 cm by 30 cm deep. Fresh algae were collected from an algal reef at Isla Margarita, near Punta Galeta. Animals were supplied with an excess (more than could be eaten in 24 hours) of the six most common reef algal species: (1) Sargassum natans (L.) J. Meyen, a large brown alga, 15 - 30 cm long, with a central axis and leaf-like blades (drift Sargassum sp. was also used as it was very abundant on the reef; no difference was observed in the response of crabs between the two species),

(2) Dictyota bayteresii Lamour., a thin, blade-like, brown alga that branches dichotomously, (3) Halimeda opuntia (L.) Lamour., a branched, calcified green alga, (4) Caulerpa racemosa (Forssk.) J. Ag., a coarsely branched siphonacious green alga, and (5) Laurencia papillosa (Forssk.) Grev. and (6) Acanthophora spicifera (Vahl) Borg, both coarsely branched red algae. Equal volumes of algae (20 ml of each) were presented in a clamp designed to give the crabs equal visibility and access to each of the algal species (some species have floats or are positively buoyant when removed from their holdfasts while others sink). Equal volumes rather than equal weights of algae were offered to crabs as this would give the crabs more equal access to the plants. As these plants differ greatly in their specific gravities, this was considered more appropriate than giving the crabs equal wet weights of algae. Weight-to-volume regressions were determined for each of the algal species so that volumes could be estimated from plant weight (Table V-1). The clamps were made of two Plexiglas rectangles (3 cm by 18 cm) padded with foam rubber and held closed with plastic cable ties. The foam padding held the plants firmly, but did not injure them. Three algal species could be placed in each clamp, therefore crabs were given two clamps with all six species. The positions of the algae in the clamps were randomized daily.

Two sets of control algae were used to assess algal losses and gains not attributable to the crabs and measurement errors. These were in the same type of apparatus, but without crabs present.

Algal weights were measured before they were offered to the crabs and again 24 hours later. Differences between control and experimental algae could be attributed to consumption by the crab. Tanks were cleaned of feces and other matter daily.

Feeding data were obtained from four females, two observed for 6 days, and two for 10 days. The crabs averaged 128 mm in carapace length (maximum anterior-posterior distance) and 977 g mass (Table V-2).

Juvenile Crab Experiments

The juvenile crabs used were reared from larvae. One crab (55) was eight months old, while the other three (60, 64 and 65) were seven months old. Although these crabs were the same ages, there was a large range in size (19 mm to 56 mm length; Table V-2). Due to the small size of the crabs, they were kept in 2 liter plastic containers with the walls and lid replaced by plastic mesh (1 cm mesh) to ensure good water circulation. These containers were kept in outdoor, flow-through, running sea water tanks, identical to those used for the adults.

The same algal species that were used for the adult experiments were used for the juveniles. Daily, equal volumes (5 to 10 ml) of each of the algae were placed in the containers with the crabs. The small size of the contained area gave the crabs equal access to all of the algae. Control algae were placed in similar containers without crabs. Weighing procedures were the same as for the adult crab experiments. The four juvenile crabs were observed for nine consecutive days.

The adult crabs were analyzed separately from the juvenile crabs. In both cases, Kendall's test of concordance was used to determine if the rank order of preference was the same among crabs (Sokal and Rolf 1981). Then a Kruskal-Wallis test was used to determine if the mean amount of each alga eaten differed, and a Student-Newman-Kuels test was used to determine which means were different from the others (Zar 1974).

RESULTS

The overall rankings of the algae from most eaten to least for the adult crabs as a function of absolute volume eaten was: (1) Caulerpa, (2) Sargassum, (3) Dictyota, (4) Acanthophora, (5) Halimeda, and (6) Laurencia (Table V-3). Although individual crabs varied somewhat in their rank order of preference, there was a significant agreement in the rank orders (Kendall's Test of Concordance, $w = 0.57$, $p<0.05$). In general, the most preferred algae were Caulerpa and Sargassum, and the least preferred were Laurencia and Halimeda (Kruskal-Wallis Test, $p<0.05$, Student-Newman-Kuels Test, $p<0.05$) (Table V-3).

The overall rank order of preference for the juvenile crabs, as a function of absolute volume eaten from most preferred to least was: (1) *Caulerpa*, (2) *Dictyota*, (3) *Sargassum*, (4) *Laurencia*, (5) *Halimeda*, and (6) *Acanthophora*. The individual rankings of the crabs varied and there was no significant agreement in the overall rankings (Kendall's Test of Concordance, w=.37, $p>0.10$). In general, *Caulerpa* and *Dictyota* were preferred over the other algae (Kruskal-Wallis Test, $p<0.05$, Student-Newman-Keuls Test, $p<0.05$; Table V-3).

DISCUSSION

Mithrax spinosissimus ate many different algal species each day. Although certain algae were consumed more than others, this crab appeared to be a generalist herbivore since when given a choice of six different algal species, it ate a mixed diet, both as a juvenile and as an adult. Two of the six algal species tested appeared to be preferred by the adult crabs, *Caulerpa racemosa* and *Sargassum natans*. *Laurencia papillosa* and *Halimeda opuntia* were eaten much less than the other four algae. The juvenile crabs also preferred *Caulerpa*, but, unlike the adults, ate more *Dictyota* than *Sargassum*. Although these were the overall general trends, different animals had different ranked preferences.

These results are not concordant with the predictions made by hypotheses about the role of defensive structure or defensive chemicals in marine algae. *Caulerpa* and

Sargassum, the two highest ranking algal species for adults, have chemicals which are suggested to have anti-herbivore effects. Caulerpa has caulerpin and caulerpicin (Doty and Santos 1968, Paul and Hay 1986), and Sargassum has tannins (Glombitza 1977, Paul and Hay 1986). Such chemicals are thought to be most effective against generalist herbivores (Fenical 1975). The two lowest ranking algae also have potential anti-herbivore chemicals (Halimeda has halimedatrial, Paul and Fenical 1983) or are related to algae which have potential anti-herbivore chemicals (other species in the genus Laurencia, and several species in the family Rhodomeleaceae, Izac 1979). Dictyota appears to have chemical compounds that are a feeding deterrent to herbivorous fishes, but a feeding attractant to amphipods (Paul and Hay 1986, Hay pers. com.).

Thallus form is also thought to protect marine algae from herbivory, but this hypothesis is not supported by the present data. Littler and Littler (1980) and Littler et al. (1983) have proposed a functional form model to predict the susceptibility of marine algae to herbivores. Their model (constructed with these genera included) predicts that Dictyota (a sheet-like alga) would be the most susceptible to herbivores, then Acanthophora, Laurencia and Caulerpa (coarsely branched species), then Halimeda (a branched calcareous alga) and finally Sargassum (a leathery alga) would be the least susceptible to herbivores. The above results are inconsistent with this hypothesis. However, the juvenile crabs did prefer *Dictyota* much more than the adult crabs. Of the six algae examined, *Dictyota* requires the least amount of force for *Mithrax* to remove tissue, but *Caulerpa* requires the most (Chapter IV). Clearly, factors other than algal structure or defensive chemicals are influencing food choice by this species.

The feeding preferences of other reef herbivores have been determined, particularly for sea urchins and some fishes (Lawrence 1975, Ogden and Lobel 1978, Hay 1984). One interesting result of these studies was the consensus that algae such as *Caulerpa* that contain potential anti-herbivore chemicals are not consumed by generalist herbivores, and are eaten primarily by specialists. Clearly, *M. spinosissimus* is a generalist herbivore, and *C. racemosa* was found to be its most preferred food in this study.

Because *M. spinosissimus* is such a large species, and can be quite abundant, it could have profound effects on the abundance and diversity of macrophytes found on algal reefs. This is particularly the case when the most preferred algae are the most abundant species, or are species that are competitively superior. On the reef at Punta Galeta, *Laurencia*, *Halimeda* and *Acanthophora* are more abundant in terms of biomass and cover of primary space than either *Caulerpa* or *Sargassum* (J. Cubit, pers. comm; however, large rafts of *Sargassum* are commonly washed onto the reef during the windy season, from mid-December through March, which was when adult feeding experiments were conducted).

Additionally, the results of these experiments indicate the importance of separating feeding preferences from potential mechanical or chemical defenses of plants against their herbivores.

Table V-1. Correlations of algal weight with algal volume for food species, where algal weight = algal volume x b + a. r equals the correlation coefficient from a linear regression. N equals sample size.

ALGA	Y INTERCEPT (a)	SLOPE (b)	r	N
CAULERPA	-0.049	0.994	0.99	10
ACANTHOPHORA	-0.018	0.832	1.00	10
HALIMEDA	-0.013	0.841	0.99	10
SARGASSUM	0.008	0.990	0.98	10
LAURENCIA	0.010	0.972	0.99	10
DICTYOTA	0.010	0.910	1.00	10

Table V-2. Average sizes of M. spinosissimus and average amounts of algae eaten.

CRAB	CARAPACE LENGTH (mm)	WEIGHT (g)	VOL. OF ALGAE EATEN/DAY (ml/d, $x^{a}(s)^{b}$)	VOL. OF ALGAE EATEN/WT OF CRAB (ml/d/g, x)
1	124	844	46.8 (9.9)	.055
2	130	1061	44.9 (10.6)	.042
3[c]	124	916	25.1 (6.7)	.027
4	135	1086	47.3 (7.6)	.044
Total				
Average	128	977	41.0	.042
(s)	(6)	(116)	(10.7)	(.012)

Juvenile Crabs:

CRAB	LENGTH (mm)	VOL. OF ALGAE EATEN/DAY (ml/d, x(s))	VOL. OF ALGAE EATEN/LENGTH OF CRAB (ml/d/mm, x)
55	34	10.6 (3.4)	0.313
60	19	2.5 (2.1)	0.128
64	40	11.1 (3.4)	0.279
65	56	12.3 (2.9)	0.219
Total			
Average	37	9.1	0.235
(s)	(15)	(4.5)	(0.081)

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[a] x = average

[b] s = standard deviation

[c] This animal had only one chela.

TABLE V-3. Average volume (ml) eaten per day of each of six algae[a]. Standard deviations are in parentheses. Bars underline means that are not significantly different (Student-Newman-Keuls Test, p<0.05).

ADULT CRAB	L	H	A	D	S	C
1.	2.76 (2.60)	7.92 (4.58)	6.32 (4.30)	10.36 (4.10)	10.69 (6.43)	10.42 (6.18)
2.	3.75 (2.59)	2.98 (2.57)	10.31 (4.20)	5.60 (5.21)	9.15 (3.22)	14.29 (6.27)
3.	1.28 (1.76)	1.31 (1.30)	5.52 (3.38)	5.27 (3.74)	6.42 (2.02)	5.30 (3.57)
4.	1.31 (0.74)	4.08 (3.70)	5.44 (3.06)	6.84 (5.02)	11.70 (2.79)	17.95 (2.19)
ALL	2.22 (2.45)	3.59 (3.91)	6.65 (4.23)	7.06 (4.78)	10.29 (4.31)	11.24 (7.02)

JUVENILE CRAB	A	H	L	S	D	C
55.	0.89 (1.19)	1.11 (0.98)	0.99 (0.90)	0.76 (0.90)	2.15 (0.67)	4.72 (2.56)
60.	0.35 (0.54)	0.12 (0.16)	0.68 (1.02)	0.29 (0.40)	0.48 (0.79)	0.54 (0.97)
64.	0.82 (0.84)	1.68 (1.42)	0.57 (1.15)	0.90 (0.70)	2.58 (0.92)	4.31 (2.59)
65.	1.35 (1.29)	0.77 (0.38)	1.28 (1.60)	2.11 (1.00)	4.32 (1.63)	2.45 (1.39)
ALL	0.85 (1.03)	0.92 (1.02)	0.95 (1.22)	1.02 (1.01)	2.39 (1.72)	3.01 (2.55)

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[a] Alga: L=*Laurencia*, H=*Halimeda*, A=*Acanthophora*, D=*Dictyota*, S=*Sargassum*, and C=*Caulerpa*.

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Chapter VI. The effect of calcification on secondary tissue loss and its implications for algal morphology.

INTRODUCTION

Recent reviews of marine plant-herbivore interactions have drawn attention to potential causal links between herbivore and plant distributions as well to correlations between the relative abundance of different marine plant growth forms and other environmental factors (Lubchenco and Gaines 1981, Gaines and Lubchenco 1982, Hawkins and Hartnoll 1983). Both Hawkins and Hartnoll, and Lubchenco and Gaines, suggested that plant form, mode of feeding of the herbivores, and chemical and structural defenses of plants all influence the resistance of plants to herbivores. However, they also indicated that not enough empirical data exist at present to draw general conclusions about the relative importance of these factors in determining plant distributions.

In an effort to construct a generalized approach to examining marine plant-herbivore interactions, Lubchenco and Gaines (1981) formulated a model, based on conditional probabilities, to determine the potential impact of herbivores on plants. Lubchenco and Gaines defined the expected herbivore damage to a plant on the basis of: the probability that a plant will be encountered by an herbivore, P(En), the probability that the herbivore will eat a plant given it is encountered, P(Ea|En), and the expected decline in fitness of the plant, relative to the rest of the population, due to the consumption of tissue by the herbivore, $E(1-w|EnEa)$. Thus the cost of herbivory would be:

$$P(En)\,P(Ea|En)\,E(1-w|EnEa)$$

where $$E(1-w) = \sum_{i=1}^{n} P(C_i)(1-w_i)$$

and $P(C_i)$ = the probability of total consumption of the ith small part of plant (approximately equal to one bite). C_i = the amount of the ith part being consumed and $(1-w_i)$ = the relative decline in fitness.

Given this model, the potential defenses of plants against their herbivores would include either temporally or spatially avoiding herbivores, minimizing $P(En)$, as well as coexistence escapes, minimizing either $P(Ea|En)$ or $E(1-w|EnEa)$. Structural and chemical defenses were considered to act primarily to minimize $P(Ea|En)$, reducing the probability of damage. If defenses are particularly expensive to the plant, or involve a major tradeoff in terms of survival or reproduction, one would predict that the defenses would be differentially distributed within the plant to minimize the expected fitness decline due to the loss of that tissue, $E(1-w_i)$.

Gaines and Lubchenco divided the marine macrophytes into seven forms: sheets or tubes, filaments, fleshy blades, fleshy branched, erect calcareous, fleshy crusts and calcareous crusts. They found different relative abundances of most of these groups with latitude, as well as differences between the east and west coasts of North America at the same latitudes. In most cases these patterns were correlated with herbivore abundance and diversity. On shores with a greater abundance and diversity of herbivores, which have been interpreted as having greater intensity of herbivory, herbivore-resistant plant forms were relatively more abundant (minimizing P(Ea|En)). These herbivore-resistant plants included the calcified plants, both crustose and erect, fleshy crustose plants and noncalcified branched plants (Gaines and Lubchenco 1982). These forms are thought to be competitively inferior to to those more vulnerable to herbivores (Wanders 1977, Vine 1974, Steneck 1982, 1983; but see Padilla 1982 and Johnson and Mann 1986a), implying a tradeoff between herbivore resistance and competitive ability.

Hawkins and Hartnoll (1983) noted similar patterns and also attributed herbivore resistance to the same algal morphologies. Other researchers have erected hypotheses predicting, among other factors, algal resistance to herbivores from plant form (Littler and Littler 1980, Steneck and Watling 1982). These patterns have been synthesized into a general view of algal morphology suggesting that plant form and calcification act primarily to increase alga mechanical resistance to herbivore damage (Littler and Littler 1980, Johansen 1981, Lubchenco and Gaines 1981, Gaines and Lubchenco 1982, Steneck and Watling 1982, Hawkins and Hartnoll 1983, Littler et al. 1983).

Mechanical tests with two important types of herbivores, however, do not support these hypotheses. I have measured the mechanical resistance of a variety of marine algae, both temperate and tropical, to several different herbivores of two important feeding types, rasping herbivores (docoglossan limpets, Chapters II and III) and biting herbivores (herbivorous crabs, Chapter IV). In both cases the results were inconsistent with these models. Neither degree of calcification nor the form of the plant appeared to influence mechanical resistance to tissue loss by these herbivores. These results imply that, at least for two important groups of herbivores, calcification and plant form are not acting as simple mechanical defenses resisting plant tissue loss to herbivores (i.e., not minimizing $P(Ea|En)$). Alternative explanations for the observed correlations between patterns of distribution of particular plant forms and herbivory must be considered.

I propose here that another major component be added to the above equation regarding the total impact of herbivores on plants they consume. This is the probability of subsequent tissue loss due to herbivore damage and its concomitant change in fitness. Adding this component to the equation of Lubchenco and Gaines yields:

Expected total impact of herbivore damage=

$$P(En)\ P(Ea|En)\ E(1-w|EnEa) + P(Sl|EnEa)\ E(1-w'|EnEaSl)$$

where $P(Sl)$ is the probability of secondary loss of a unit of plant tissue due to the damage and $E(1-w')$ is the expected change (either increase or decrease) in fitness due to this additional tissue loss. The fitness decrement due to tissue lost secondarily may be very different from that due to direct consumption by an herbivore.

For some algal species, secondary tissue loss may greatly exceed primary loss to herbivores (Black 1974, Santelices et al. 1980, Johnson and Mann 1986b). Black (1974) found that of the Egregia laevigata (Phaeophyta) plants washed on the shore in southern California, 122 of 129 broken rachises, and 58 of 86 broken stipes were attributable to herbivore damage. Johnson and Mann (1986) found that although the snail Lacuna vincta ate only 0.05% of the available biomass of Laminaria longicruris (Phaeophyta), the total consequence of secondary tissue loss cost these plants 27.3% in lamina area increase and 28.3% standing biomass as compared to ungrazed plants. Clearly, secondary tissue loss needs to be assessed when measuring the impact of herbivory.

I would also like to suggest that calcification and other potential "structural defenses" such as thallus form play a more important role in minimizing this secondary tissue loss (minimize P(Sl|EaEn)) than in preventing herbivores from removing tissue (minimizing P(Ea|En)) as has been suggested in the past.

Plants that are crustose, branched and calcified may be less susceptible to secondary tissue loss than are other algal forms when subsequently subjected to stresses (shear and drag) of water motion. Although tissue is still lost to herbivores, the total impact of herbivory is reduced. Secondary tissue loss may be a more accurate descriptor of the impact of herbivory if it far exceeds the losses due to direct consumption by herbivores (if E(1-w') >> E(1-w); Black 1976, Johnson and Mann 1986b). If a complex, quantitative trait (like plant form) has a strong selection gradient imposed by one factor (resistance to secondary tissue loss) and a weaker selection gradient imposed by a second factor (mechanical resistance to herbivore damage) then selection would act on this trait primarily in response to the stronger selection gradient (Lande and Arnold 1983). In this case, if a particular plant trait reduced secondary tissue loss more effectively than it reduced loss to herbivores directly, it would be favored by selection over a trait whose primary effect was to reduce direct tissue loss.

Plants could minimize secondary loss in several ways, including having large breaking strengths or by preventing tissue damage from subsequently weakening the structure of the plant. To test whether calcification could increase plant structural strength and prevent the propagation of damage in marine macrophytes, I measured the breaking strengths, in tension, of a variety of plants with varying degrees of calcification. If calcification generally increases plant strength one would expect an increase in plant strength with increasing degrees of calcification. The breaking strengths of herbivore-damaged and undamaged plants were also compared to determine if herbivore damage reduced plant strength. The position of the point of breakage was also noted to determine if the point of herbivore damage was the point at which the thallus broke. Finally, experiments were conducted to determine if loss rates differed between damaged and undamaged plants as a function of degree of calcification.

METHODS

This research was conducted at the Smithsonian Tropical Research Institute marine laboratory at Punta Galeta (Chapter III). Algae were collected from shallow, algal reefs, the Galeta Lab reef and similar reefs to the west at Isla Margarita.

Breaking strengths were measured with a minitensometer. Each end of the plant was placed in a padded grip that could be tightened, so the plant did not slip when placed in tension, but that did not damage the plant. Plants which were particularly difficult to hold were attached to the grips with a cyanoacrylate adhesive. The padding on the grips was made of hot-melt-glue (Thermogrip Inc.). The two sides of the grip were matched by placing a piece of sheet teflon between them, closing the grip tightly and placing it in very hot water until the two sides had conformed to a smooth surface. Small pieces of sheet silicon were placed in the back portions of the grip so that the grip placed an even force across the thallus irrespective of thallus shape or size. Trials in which algae broke at the grip were not counted. One grip was fixed to a force transducer mounted at one end of the minitensometer. The other was fixed to a slide which moved along a threaded rod. The rod was rotated with a small hand crank at one end. A linearly variable differential transformer (LVDT) was also attached to the moveable slide. Thus, once a plant was placed in the grips, the crank was turned placing the plant in tension. The plant was strained at a constant rate, with the deflection and forces required simultaneously recorded on a chart recorder. All plants were submerged until used and kept moist during the trials.

The cross-sectional area of the plant at the point of breakage was determined in one of two ways. For large plants the height and width of the cross-section were measured with an ocular micrometer under a dissecting microscope at the highest power that was possible to see the entire surface. For the plants which were smaller or quite thin, the plant was sectioned with a freezing microtome. This section was then measured for height and width with the ocular micrometer of a compound microscope or dissection scope, whichever was appropriate. Cross-sectional shapes were considered to be either rectangles or ellipses, with the area determined appropriately for each. Each area was measured three times, and the error was less than three percent.

To measure the difference in the breaking strengths between damaged and undamaged plants, I presented undamaged plants to a Stoplight Parrotfish (Sparisoma viride) which was kept in an aquarium. Therefore, the same individual herbivore bit all of the plants tested to control for possible differences in bite shape, size or biting technique. The breaking strength of the damaged plants was then measured and compared to the undamaged plants of the same species. The actual point along the thallus at which breakage occurred was also determined to test whether breakage occurred preferentially at the point of damage.

Finally, the loss rates of damaged and undamaged plants in the field were measured. Plants were exposed to the same captive parrot fish that was used in the breaking strength experiments. These damaged plants were then paired by visual assessment with undamaged plants of approximately the same size and shape. Pairs of plants were then placed in Plexiglas holders, and attached with biosponge, a polymer adhesive, which attached the plants to the Plexiglas, padded them but did not damage them. These were then attached with plastic cable ties to a $1m^2$ piece of plastic mesh attached to the reef surface with steel spikes near the wave exposed reef edge. Plants were measured, photographed and placed out as the water was rising on days of heavy wave action, were left out while the water was high and removed as the water began to recede, to minimize the probability of herbivores grazing on the experimental plants. Hence any plant tissue loss could be attributed to wave and current stress rather than to herbivores. The plants were measured and photographed again after removal and tissue loss was determined by the change in height of the plant.

RESULTS

Calcification does appear to increase plant strength. Six noncalcified algae (Acanthophera spicifera, Laurencia papillosa, Dictyota bayteresii, Caulerpa racemosa, Caulerpa sertuleriodes, and Anadyomene stellata), three calcified algae (Padina jamacensi, Halimeda opuntia, and Amphiroa hancockii), and an angiosperm, the sea grass Thalassia testudinum, were compared. Algae which were calcified were significantly stronger than those which were not calcified (Oneway analysis of variance, $p<0.01$, Statgraphics, STSC, Inc. 1985; Student-Newman-Kuels test, $p<0.05$; Zar 1974). The breaking strength of the sea grass was within the range of that of the calcified algae (Figure VI-1).

Three noncalcified plants (Dictyota, Acanthophora, and the sea grass Thalassia) and the three calcified plants were also tested to determine if herbivore damage reduced plant strength. For the seagrass and one of the two noncalcified algae, plant strength was significantly reduced by herbivore damage (Mann-Whitney Test, $P<0.05$; Zar 1974). There was no significant difference in breaking strength between damaged and undamaged plants for any of the calcified plants (Mann-Whitney Test, $P>.1$; Zar 1974) (Figure VI-2). Additionally, the calcified plants were less likely to break at the point of damage than were those which were not calcified (Figure VI-3). All of the noncalcified plants of each of the two algal species tested and the seagrass broke at the point of herbivore damage. For the calcified algae, most broke at points other than the place of damage. These differences are significant (Mann-Whitney Test, $p<0.05$; Zar 1974).

The field experiments on loss rates supported these results as well. Although it was not possible to obtain data for noncalcified algae due to difficulty with the holding apparatus and limited time, the sea grass and three species of calcified algae were tested. There was no significant difference in the loss of tissue as a function of herbivore damage for the calcified plants (Mann-Whitney Test, $p> 0.8$, Zar 1974). This was not the case for the seagrass ($p<0.01$). Seven of 8 seagrasses lost height while only 1 of 14 calcified plants lost height. No controls lost tissue (Table VI-1).

DISCUSSION

The general model of Lubchenco and Gaines (1981), assessing the impact of herbivores on plants, includes the probability that a plant is encountered and and the probability that it is subsequently eaten by an herbivore. This suggests that chemical, structural and nutritional attributes of a plant influence the probability of tissue consumption by the herbivore. The decline in fitness of a grazed plant relative to undamaged plants would depend on tissues eaten (holdfasts, meristems), season of damage (whether the plant was reproductive or not), and the ontogenetic stage of plant at the time of damage (a sporeling versus mature versus post-reproductive plant). I suggest that in addition to the direct tissue losses to herbivory, this decline in fitness potential should include the probability of secondary tissue loss following damage to the thallus by herbivores.

Calcification may make plants stronger, more resistant to breakage, and more resistant to tearing (the propagation of critical cracks, Gordon 1968) in plants that are already damaged. Cracks or tears in materials generally cause them to break when tensional forces are placed on them (Wainwright et al. 1976). The probability that any damage will create a critical crack (one that leads to failure with no further input of tensional stress) will depend both on the amount of force on the material, and exact size and shape of the damage. Discontinuities and weak interfaces in the material can prevent crack propagation (Wainwright et al. 1976, Gordon 1968). Discontinuities force the crack to move around weak interfaces, dispersing crack energy, preventing crack propagation. Calcification in marine plants could have this crack stopping capacity. In this way, a calcified plant damaged by herbivores would be less susceptible to secondary tissue loss than would a similarly damaged noncalcified plant.

A similar argument may be developed for overall plant morphology. Sheet-like plants are those generally considered to be the most susceptible to herbivores. If an herbivore takes a bite or damages a sheet-like alga, the crack or tear made can propagate across the entire thallus resulting in great tissue loss. The location of damage will determine how much and what tissue will be lost. Although the amount of tissue actually consumed by the herbivore may be quite small, the loss of tissue due to the damage may be quite large. Branched plants are considered more resistant to herbivores than sheet-like forms (Littler and Littler 1980, Lubchenco and Gaines 1981, Gaines and Lubchenco 1982, Steneck and Watling 1982). By dividing the thallus into branches, damage to any single branch will not affect the other branches. Although a plant of this morphology may have equal or less mechanical resistance to the herbivore, the consequences of the damage imposed by the herbivory will be limited to the immediate branch affected, restricting the overall impact of herbivory. For an encrusting plant the negative consequences of herbivory will be limited to the amount of tissue actually consumed by the herbivore. The thallus is completely attached to the substratum on one side, therefore, any herbivore damage will not make the rest of the thallus susceptible to secondary loss. Thus, many algal traits including calcification, dividing the thallus, or being crustose, could reduce the probability or amount of tissue lost due the mechanical stresses of water motion following any type of damage that a plant might experience, whether imposed by herbivores or other sources (e.g., abrasion, rocks, logs, etc).

For marine plants protection from tissue loss once damaged will be important even if they possess other types of anti-herbivore defenses. Frequently, anti-herbivore chemicals in plants are hydrophobic, therefore herbivores must taste the plant, thereby damaging it, for these chemicals to be effective (Norris and Fenical 1982, Hay pers com). Also, the nutritional qualities of the plant may be most easily assessed from taste, again imposing damage on the plants. Hence, even if the plant has alternative defenses against an herbivore, protecting the thallus from secondary tissue loss due to damage could be very important. This could explain why plants, such as Halimeda, which have potent anti-herbivore chemicals are also calcified (Paul and Fenical 1983, Paul and Hay 1986).

The results of the experiments described above suggest that calcification, and by inference overall form, in marine plants could be mechanisms which minimize the probability of secondary tissue loss following damage, including that caused by herbivores. Calcification strengthens and prevents tearing where the plant has been damaged. For the plants tested, this mechanism appears to be effective in the field. Although I was unable to test field loss rates for the noncalcified algae, the forces required to break damaged plants were less than those for calcified plants and Thalassia. Because the latter did lose tissue under the conditions of the field experiment, forces sufficient to cause secondary tissue loss in noncalcified algae probably did occur. Clearly more experiments are needed to test the generality of these results, particularly for plants of different thallus form.

These results may also be relevant to the evolution and maintenance of calcification in marine plants. Several different explanations have been proposed to account for the evolution and maintenance of algal calcification: calcification may be a byproduct of photosythesis in calcium saturated water (Borowitzka 1982), it may be a means of detoxifying the cells (Simkiss 1977, Brasier 1986), or it may be a major structural deterrent to herbivores (Johansen 1981, Steneck and Watling 1982, Steneck 1982, 1983, Hawkins and Hartnoll 1983). Calcification could also be selected for or maintained for protection against herbivores which are not capable of eating calcium carbonate, or for protection from abrasion along the thallus edge. It could also be important in influencing the wear rates of the feeding apparatus of an herbivore and therefore affecting feeding efficiencies of herbivores, or affect digestive efficiencies in animals with acidic digestive systems.

Clearly all algae do not calcify, so one might ask the question why; what would be the selective disadvantages of calcification? One disadvantage might be that calcification slows growth rates. Some calcified algae grow slowly (1-2cm/yr, Johansen 1981); however, some heavily calcified algae in the tropics grow very rapidly (1-2cm/week, Padilla pers. obs.). Therefore growth rate may depend on the type of calcification (Borowitzka 1982) and local environmental conditions. Also, calcification makes plants more susceptible to desiccation (Johansen 1981, Dromgoole 1980), and may make them more susceptible to certain types of herbivores (Chapters II and III).

Ironically, the patterns of abundance and distribution of marine plants and herbivores that would be observed if calcification and thallus form acted as direct defenses against herbivory or were acting to minimize secondary loss would be the same. Therefore, does knowing the exact mechanism matter? The answer will depend on the question in which one is interested. At the ecosystem level the answer is yes if one wishes to know where energy is flowing (e.g., carbon and nitrogen budgets). At the plant community organization level the answer is probably no. The consequences of damage on the distribution and abundance of the plants would be the same independent of exact mechanism. They may differ quantitatively, but not qualitatively. At the population level the answer again would be yes, particularly regarding the potential for selection on, and effectiveness of anti-herbivore defenses. A plant could suffer greater tissue loss directly to herbivores, but have a much lower total fitness reduction than plants with other forms due to minimizing secondary tissue loss.

Table VI-1. The number of damaged and undamaged that lost tissue (=change in plant height) in the field. Table entries are the number of plants that lost tissue over the number of plants tested.

Plant	Damaged	Undamaged
Seagrass:		
Thalassia	7/8	0/8
Calcified alga:		
Udotea	0/4	0/4
Padina	1/4	0/4
Halimeda	0/6	0/6

Figure VI-1. Breaking strengths (MN/m^2) of calcified and noncalcified algae and the angiosperm Thalassia. Bars represent averages and lines indicate the standard errors. Sample sizes are above the bars.

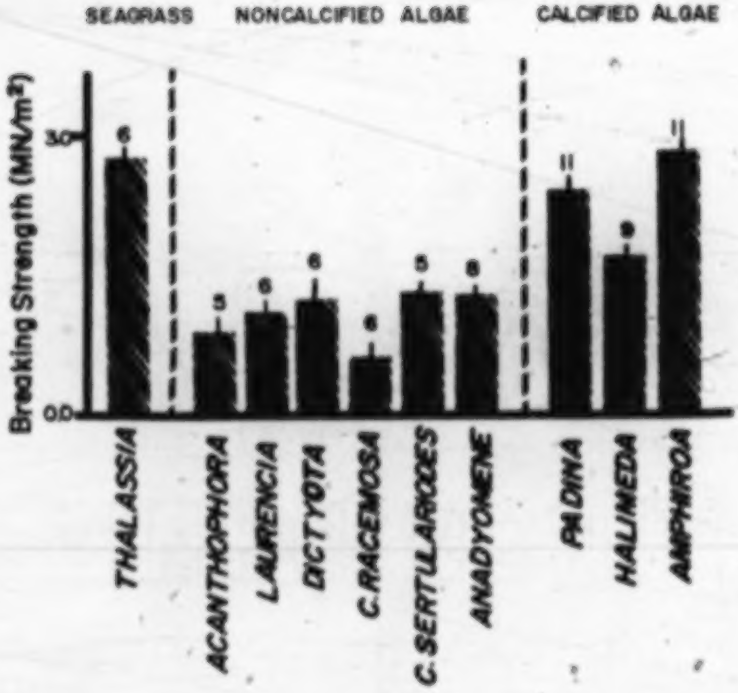


Figure VI-2. Breaking strengths (MN/M^2) of herbivore damaged vs undamaged plants. Bars represent averages and lines indicate the standard deviations. Sample sizes are above the bars.

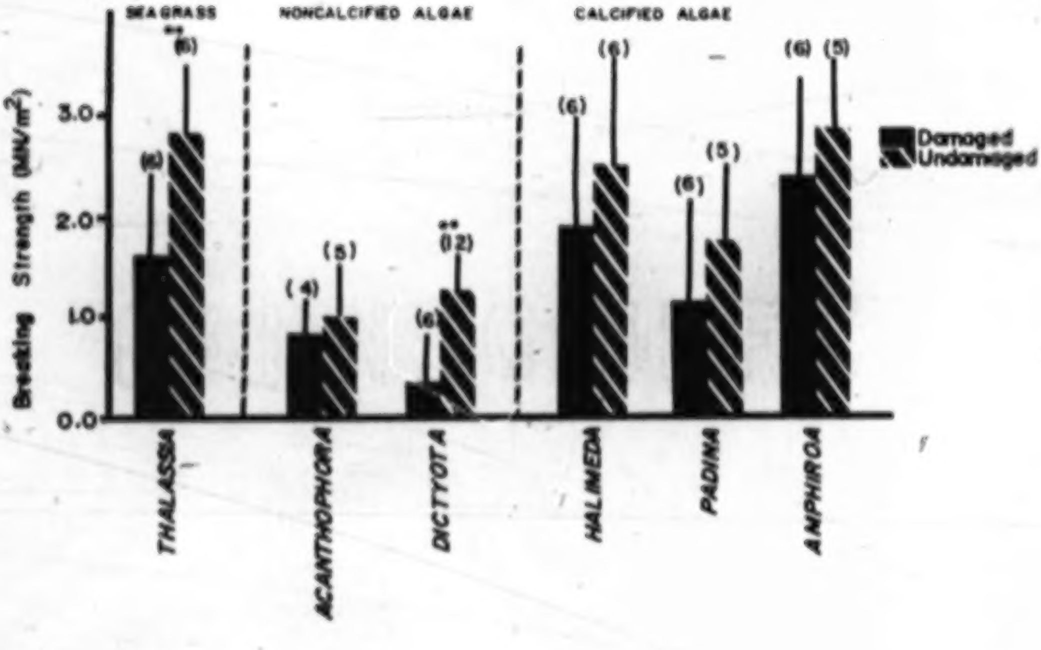
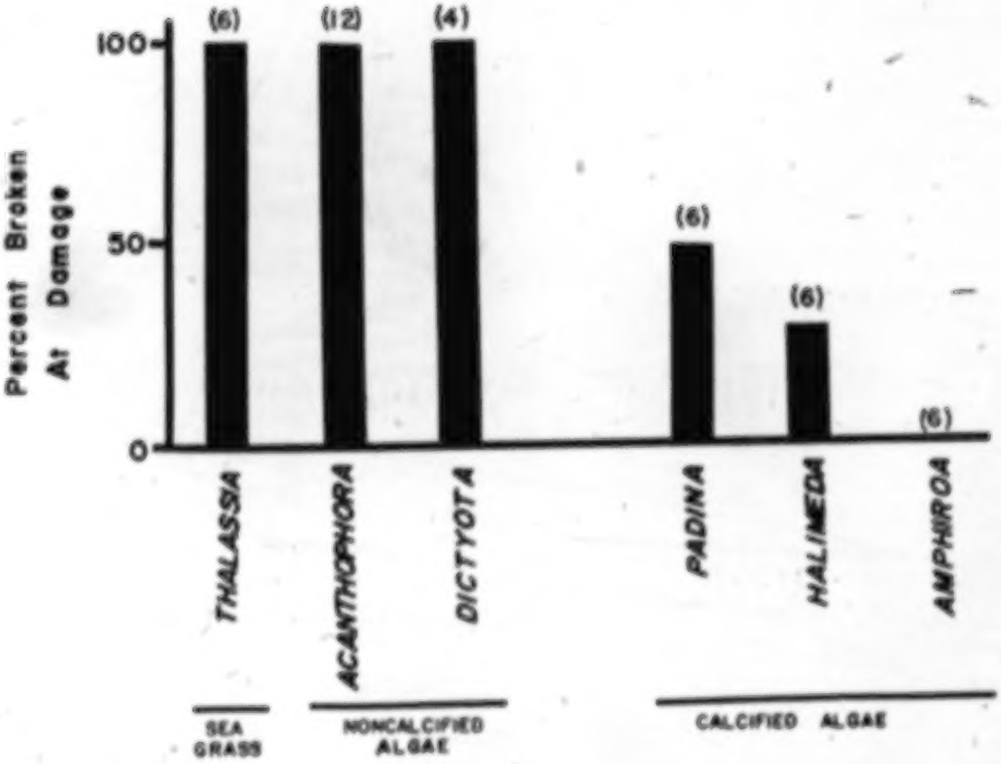


Figure VI-3. Percent of damaged plants which broke at the point of damage. Sample sizes are in parentheses.



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Chapter VII. Conclusions.

The results presented in chapters II, III, and VI reveal that structural and material properties of marine algae do affect their relative susceptibilities to tissue loss to docoglossan limpets, however, not in the ways predicted by Littler and Littler (1980) and Steneck and Watling (1982). For the temperate and tropical macrophytes examined, mechanical resistance to tissue removal by docoglossan limpets decreased with increasing calcification. Also, mechanical resistance to tissue loss was contrary to the predictions based on thallus form (Chapters II and III). Tooth shape and number appear to be important factors influencing the amount of force required for different limpet species to remove tissue from different algae (Chapter II).

Resistance to one type of herbivore, however, does not imply that a plant will be equally resistant to another type of herbivore feeding in another way. The patterns of mechanical resistance of tropical algae to the herbivorous crab Mithrax spinosissimus did not support the predictions of the functional form or functional group models, and also did not correspond to those for the limpets. Relative susceptibilities of algae to tissue loss did not relate to degree of calcification or thallus form for Mithrax (Chapter IV). In addition, the probability that a particular force applied by the chela of a Mithrax would result in tissue loss for a particular algal species varied considerably. This may be due to the high variance in the structural properties of individual algal plants.

The feeding preferences of Mithrax are different from those predicted on the basis of mechanical resistance to tissue loss or those expected on the distribution of potential anti-herbivore chemicals. The most preferred foods of adult Mithrax were Caulerpa and Sargassum, both of which not only contain anti-herbivore compounds (Norris and Fenical 1982) but also were among the algae requiring the most force for tissue removal. This underscores the importance of separating the mechanical resistance of plants to tissue loss from the feeding preferences of herbivores when examining structural defenses of plants.

Although algal calcification and plant form do not appear to influence algal mechanical resistance to herbivores in the fashion predicted by other authors (Littler and Littler 1980, Johansen 1981, Lubchenco and Gaines 1981, Gaines and Lubchenco 1982, Steneck and Watling 1982, Hawkins and Hartnoll 1983, Littler et al. 1983), they may be important in reducing the impact of herbivores on algae by minimizing secondary tissue losses subsequent to herbivore damage. Calcification makes plants strong, and more resistant to tissue loss once damaged (Chapter VI). Similarly, some plant forms, are more resistant to secondary tissue loss, such as encrusting plants or branched plants, than are sheet-like plants or blades. This type of mechanism would be important even if a plant possesses alternative defenses. Frequently, anti-herbivore chemicals are hydrophobic, requiring that the plant be tasted for these chemicals to be effective (Paul and Hay 1986, Hay, pers. com.). Also, nutritional qualities of a plant may be most easily assessed from taste, again imposing damage on the plant. This could explain why some plants which have potent anti-herbivore chemicals, such as Halimeda, are also calcified (Paul and Fenical 1983, Paul and Hay 1986). Hence, even if a plant has alternative defenses against herbivores, protecting the thallus from secondary tissue loss due to damage could be very important.

Branching patterns and thallus form are features which also influence many other aspects of macrophyte biology. Patterns of thallus form will influence the overall surface area of a plant, which will be important for nutrient uptake and reproduction, as well as the projected area of the thallus which will be important for photosynthesis. Thallus form will also influence the drag forces that a plant will experience in moving water, as well as the velocities at which laminar flow becomes turbulent over the thallus (Wheeler 1980 and in prep). For macrophtyes there is an apparent trade-off between diffusion of nutrients to the surface of the plant and drag. Photosynthetic rates can be limited by the diffusion of nutrients from the surrounding water through laminar boundary layers surrounding the thallus. Thus, a plant will increase the diffusion of nutrients if its morphology results in turbulent flow next to the thallus at lower water velocities. However, this increases the drag forces on the plant and will increase the probability of dislodgement (Denny et al. 1985, Wheeler in prep.).

Calcification may also have other consequences for algae and algal-herbivore interactions. Calcification gives erect plants enough structural integrity to allow them to stand upright, rather than lying on the benthic surface. This would prevent self shading, keep the plant above the boundary layer of the benthic surface, and perhaps protect the plant from herbivores which move on the benthic surface or require a plant large enough to crawl on (Padilla 1984). Calcification could also increase wear on the feeding apparatus of the herbivore. This could affect the feeding efficiencies of the herbivores or limit the amount of time they could spend feeding depending on the relative rate of wear and replacement of the feeding apparatus.

Given these patterns and alternative consequences of plant form and calcification, it appears as though these features do not act primarily as deterrents to herbivores. Clearly, indirect and sometimes subtle factors are influencing the interactions between marine plants and their herbivores, and the importance of these factors in influencing evolutionary patterns of plant form and calcification is not known. There is an extensive fossil record of calcified algae, however, fossils of non-calcified algae are rare. It is therefore difficult to separate the evolutionary patterns of plant form from those of calcification (Flugel 1977). Steneck has examined rates of speciation in the encrusting species of the Corallinales (Steneck 1984). He found correlations between patterns of speciation and the abundance of herbivorous taxa with excavating abilities (mineralized feeding apparatus), and concluded that the high herbivore pressures and relative resistance of calcified crusts gave them an advantage over other plants that led to rapid speciation. It would be interesting to compare these patterns with those for other calcified algae including articulated coralline algae. The articulated and encrusting coralline groupings are based only on morphology, and phyletic groupings include both growth forms (Johansen 1981). As calcified algae compose about 11% of all modern species of algae, cross taxa comparisons could elucidate general patterns and possible benefits to calcification in general.

Questions of the evolution of plant form and major selective forces influencing form still remain. Superimposed on these questions are the relative importance of changing form in marine plants. Two major modes of modifying plant form are phenotypic plasticity and heteromorphic life histories (Mathieson et al. 1981). Phenotypic plasticity allows a plant to change qualitatively, and within a short time period, while heteromorphy results in qualitative changes and require life stage changes as well. Comparisons of species along a continuum of morphological flexibility may reveal factors that have major importance in the evolution of plant form.

To date much of marine plant-herbivore ecology has been based on patterns in geographic distribution and community structure and organization. A more focused examination of specific interactions and the mechanisms of interactions should help shed light on these more general problems.

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Appendix 1. The effect of growth environment on morphological variation in an articulated coralline alga, Calliarthron tuberculosum

INTRODUCTION

Morphological variation among populations can be observed in many different types of organisms, particularly along environmental gradients. This variation among populations can be due to genetic differences or it can be a phenotypic response to different environmental factors. A phenotypic response can be adaptive (= plastic, Bradshaw 1965, Smith-Gill 1983) or it can be non-adaptive, perhaps even being beyond the organism's control.

The form of many marine macroalgae is highly labile, and this lability is particularly apparent along environmental gradients (Mathieson et al. 1981, de Paula and de Oliveira 1982, Norton et al. 1983, Payri 1984). Morphological variation has been studied in marine macrophytes because of its importance in establishing taxonomic identities of various morphologies as well as linking pleomorphic life histories (Mathieson et al. 1981). In addition, morphological variation in marine plants has been correlated with a variety of environmental factors including temperature, photon flux, wave exposure, desiccation and herbivores (Mathieson et al. 1981, Lewis et al. 1987).

Calliarthron tuberculosum, an articulated coralline red alga, lives in large tidepools, low in the intertidal, and in shallow subtidal zones along wave swept shores in the eastern Pacific from Alaska to California, and varies phenotypically along environmental gradients, including along wave exposure gradients (Johansen 1969). Calliarthron also appears to have very local spore dispersal and therefore the potential for local genetic differentiation among populations (Padilla 1981, 1984).

Cross-transplant experiments have been suggested to be one of the most effective means of determining if phenotypic differences in a seaweed are environmentally or genetically determined (Chapman 1978, Mathieson et al. 1981). However, if one cross-transplants established plants it is difficult to interpret the results as growth conditions early in life may influence subsequent form or there may be a long lag time for morphological change in an established plant (Chapman 1974, 1978). Therefore, I attempted reciprocal transplant experiments with sporelings to determine the degree to which different environmental factors influence growth form in Calliarthron. Specifically, the effects of both wave action and herbivory were examined by transplanting sporelings between areas of differing wave exposure and by allowing plants at each site to grow either with or without exposure to herbivores.

In wave swept environments plants experience high drag forces that can dislodge them (Denny et al. 1985). Therefore, plants in this type of environment would be expected to have a structure that would minimize drag. In lower flow environments diffusion of nutrients to the plant surface can be a problem due to large boundary layers established around the plants (Wheeler 1980, Wheeler and Neushul 1981, Norton et al. 1983, Wheeler in prep.). Therefore, one would predict that plants in this situation would have a structure that maximizes drag, thereby reducing the boundary layer and increasing the rate of nutrient diffusion to the plant surface. As Calliarthron grows in large tide pools this phenomenon could be very important. Although the water velocities would be high when waves are hitting a pool, the plants would spend large amounts of time in still or slow moving water, particularly higher on the shore and on less wave exposed sites.

MATERIALS AND METHODS

STUDY SITES

Experiments were conducted in Barkley Sound on the West Coast of Vancouver Island in British Columbia, Canada (Figure 1-1). Within Barkley Sound wave exposure, and herbivore density and diversity may differ between sites even in close proximity. The experiments in this study were conducted at a relatively protected site (Bluestone) and two exposed sites (Prasiola Point and Prasiola Low) approximately 300 meters from the protected site.

Relative exposure was assessed by the tidal height and width of the Balanus glandula zone. This zone will be higher on the shore and wider in more exposed areas than less exposed areas (Table 1-1; Stephenson and Stephenson 1972). The height on the shore was measured once, from the water level, on a calm day, at a particular time. The actual height of the water above tidal datum at that time could then be determined from tide tables for that day.

STUDY ORGANISMS

Calliarthron tuberculosum is a long-lived perennial articulated coralline red alga with an isomorphic life-history. It has an encrusting holdfast that rarely extends beyond the bases of the upright branches. The branches are constructed of a series of calcified segments (intergeniculae) connected by noncalcified joints (geniculae) and can be up to 15 cm in length. *Calliarthron* is susceptible to herbivores primarily when it is small, during sporeling and juvenile stages. In the system studied here, the major herbivores on articulated coralline algae are molluscs (Padilla 1984).

The major herbivorous molluscs sympatric with *Calliarthron* included two species of limpets (Patellogastropoda), *Tectura* (= *Notoacmea*) *scutum* and *Acmaea mitra*, and two species of chiton (Polyplacophora), *Tonicella lineata* and *Katharina tunicata*. *Tectura scutum* is a generalist herbivore, 2 - 4cm long, and occurred at both exposed sites and the protected site. *Acmaea mitra*, a coralline algal specialist, is 2 - 3cm long and occurred only at the two more exposed sites. *Tonicella lineata* is a coralline algal specialist, 2 - 4 cm long, and occurred at the protected and at both exposed sites. Katharina tunicata is a generalist herbivore, 7 to 12cm long, and occurred only at the two more exposed sites.

In addition to the molluscan herbivores, the purple sea urchin Strongylocentrotus purpuratus was abundant at one exposed site, Prasiola Low; it did not generally occur in tidepools in Barkley Sound. It is a generalist herbivore and coralline algae are least preferred food items (Paine and Vadas 1969, Vadas 1977). Other herbivores observed occurred in low densities (Table 1-1).

MORPHOMETRIC ANALYSIS OF EXPOSURE MORPHS

To assess the degree to which Calliarthron differed among sites, a discriminant analysis was performed on a variety of morphological characters of established plants from each site (BMDP; Dixon 1983). The characters that were used for these plants included base and total height, intergenicular dimensions (height, width, and depth), degree of branching (none, primary, secondary or tertiary), and dimensionality of branchings (two or three dimensional) (Figure 1-2). These same characters, except the branching features, were used for the sporelings. All of the intergeniculae in the base of the plant were measured and an average value for each dimension was then used for the analysis. An analysis of covariance (Number Cruncher Statistical System, Hintze 1985) indicated that there was no correlation between the position of an intergeniculum and intergenicular dimensions (Table 1-2). As the plants used in this analysis had different numbers of intergeniculae (5-15), the lack of a "plant" effect indicates that total size had no effect on intergenicular dimensions.

TRANSPLANT EXPERIMENTS

In February, March and April of 1982 and 1983 10 to 15 plants were collected at each of three experimental sites to obtain spores. Single thalli were isolated in glass dishes with filtered (to 1 µm) sea water, and spores from each parent were settled onto small Plexiglas plates approximately 3 by 6cm. The plates had been roughened with sandpaper to aid spore attachment and predrilled so they could be attached to larger plates which could be placed in the field easily. Algal thalli and settling plates were kept several days at 10°C in the dark until spores were released and attached to the plates. Unfortunately, plants from Prasiola Low never released spores during the course of these experiments. Therefore, transplants were conducted only with sporelings from Prasiola Point and Bluestone parents. The spore plates were maintained in incubators with lights on the natural photoperiod and at 10°C until they could be transplanted into the field (3 to 6 weeks). They were kept in filtered sea water (to 1 µm) which was changed every two to three days. Spore plates were transplanted during June, July and August of the year in which they were collected.

Before transplanting, spore plates were examined for spore viability and growth in the lab. The number of spores per plate ranged from 6 to 70. Sporelings were photographed through a dissecting microscope (Wild camera attachment, with 10x ocular and either 12x or 25x magnification, and illuminated from the side with focused, fiber optic light sources) and mapped such that each could be identified when harvested. Each plate was placed in a small petri dish with sea water over a grid. The lower right corner of each plate was then filed flat to insure a consistent orientation of the plate on subsequent observations. The coordinates on the grid of each photograph were recorded. When plates were subsequently examined, the coordinates were located and the small scratches on the plate surface were used as features to match between the plate and the photograph to determine if a given sporeling had survived. This also made it possible to determine if any new spores had settled during the course of the experiment.

Four to twelve replicate plates of sporelings from each parent were assigned in a stratified random design to the following treatments: more exposed (2 sites) or less exposed (1 site), with or without molluscan herbivores, and a control for molluscan herbivore exclosures. Within the exposed treatments, plates were assigned to the site either with (Prasiola Low) or without (Prasiola Point) urchins. In all cases the small plates were attached with stainless steel screws to larger Plexiglas plates that had been predrilled with matching holes. The larger plates were attached to the rock substratum with a marine epoxy putty (Z-Spar Splash Zone Compound, Kopper Co.), smoothed along the edges, giving the herbivores access to the plates. All transplant locations were either inside large pools containing Calliarthron (Bluestone and Prasiola Point) or in the shallow subtidal among Calliarthron plants (Prasiola Low). The tidal heights of the shallow subtidal site (Prasiola Low) and the bottom of the Prasiola Point pools were the same (-0.1m, where 0= Canadian tidal datum, approximately lowest, lower water). There were two to four large plates per treatment at each of the three sites.

The herbivore exclusion treatment plates were surrounded by strips of sheet copper (0.2cm thick and 2.5cm wide) puttied smooth against the substratum so they did not interfere with water flow (modified from L. Johnson, in prep). The molluscan herbivores would not walk across these copper barriers. The sea urchins, however, moved freely across the copper barriers. Therefore, any differences in the structure of plants from the molluscan exclusion treatments between Prasiola Low (with urchins) and Prasiola Point (without urchins) could be attributed to the sea urchins. To control for the effects of the copper, control treatments had identical copper strips on two sides leaving the other two accessible to herbivores. Juvenile herbivores settled onto plates in all treatments and were removed from the herbivore exclosures when observed during biweekly examinations from April through September, and less frequently during the rest of the year. Transplants remained in the field for approximately one to two years. All plants were harvested at the end of August 1985.

At the end of the experiment, various morphometric measurements were made on transplanted sporelings. The same features except for branching patterns were measured on sporelings as adult plants. The transplanted sporelings had not grown sufficiently to compare branching patterns. Discriminant analysis (BMDP; Dixon 1983) was used to compare the similarities of the morphologies of parental, transplant and local populations, as well as among experimental treatments. Multivariate analysis of variance or multivariate T-tests (BMDP; Dixon 1983), using all of the same characters that were used in the discriminant analysis, were then used to test whether differences among populations or treatments were significant.

THE INFLUENCE OF FORM ON DRAG

The drag forces on 16 plants of different sizes from Bluestone and Prasiola were measured. Each plant was attached with a clip to a force beam and then placed in a flow tank such that the base of the plant was at the water surface. The water was circulated at a constant velocity, and drag force was determined after steady flow had been obtained. Drag was measured four times at each of three different flow velocities, 0.08 m/s, 0.27 m/s and 0.42 m/s. The force beam was calibrated by suspending free weights from the beam.

The length and surface area of each of the plants in the drag experiment were determined. Length was measured as the maximum linear distance from the base to the tip of the longest branch. Measurements were repeated four times and the average was used as the length of the plant. To estimate surface areas, pieces of thin plastic of known surface areas were used to establish a regression equation for weight gain/ surface area. The plastic pieces were dipped in alcohol and then weighed. They were then dipped in a surfactant (a 5% detergent solution) and weighed again. This was repeated four times for each plastic piece. The weight gain would be correlated with the surface area in a linear fashion. Each plant was dipped in alcohol and then weighed, dipped in the surfactant and weighed again. This was repeated four times for each plant and the average was used as an estimate of surface area (Hicks 1977). The accuracy of this technique was tested with a thin, bladed alga, Dictyota bayteresti, whose surface area could be directly measured. The estimate and the measurement differed by less than 1% (n=10).

RESULTS

MORPHOMETRIC SEPARATION OF TWO POPULATIONS

Using discriminant analysis the samples of Calliarthron from the protected site and from the exposed site show little overlap (BMDP; Dixon 1983). None of the Prasiola

Point plants was misclassified, but one Bluestone plant was misclassified as a Prasiola plant. Three plants from the Prasiola Low site were then used to test this separation. All of the Prasiola Low plants fell with the Prasiola Point plants along the canonical variable axis (Table 1-3, Figure 1-3). A multivariate T-Test of these data indicate significant differences between the Prasiola and Bluestone populations (Mahalanobis D^2 = 10.8232, Hotelling T^2 = 56.693, p=0.0102; BMDP, Dixon 1983).

TRANSPLANT EXPERIMENTS

At Bluestone, none of the transplants or natural recruits of Calliarthron grew large enough to have intergeniculae in either year, although adult plants in that population did grow during that time period (10 marked plants grew an average of 1.3 cm/yr). At Prasiola Low and Prasiola Point several plants did grow. However, there were too few plants among the different treatments to conduct a proper analysis (Table 1-4).

Preliminary analyses were conducted on those plants with intergeniculae transplanted to Prasiola Point and Prasiola Low. When the experiment was terminated the sporeling plants had not grown large enough to assess branching features. Thus, features demonstrating the main differences in form among the parents were not available for analysis in the sporelings.

Discriminant analysis was used to compare the structure of the transplants whose parents were from Prasiola Point, the transplants whose parents were from Bluestone and each of the parental populations. The form of the surviving sporelings more closely resembled that of the local population rather than that of their parental population if it was different (Table 1-5, Figure 1-4). The Prasiola parents and both groups of sporelings completely separated from the Bluestone plants. A multivariate analysis of variance indicated significant differences among these four groups of plants (Hotellings generalized T-zero squared= 196.416, Tiku's approximate chisquare, a test for significance of Hotellings generalized T-zero squared, = 139.41, DF= 13.923, p=0.00; BMDP, Dixon 1983). A multivariate T-Test indicated that the form of transplants whose parents were from Prasiola were significantly different from those whose parents were from Bluestone when transplanted into the same environment (Mahalanobis D^2= 2.6096, Hotelling T^2= 35.3092, F value= 4.5713, DF= 7, 58.0, p= 0.0004). This suggests that there is both a genetic and an environmental component affecting the form of these plants.

When sporelings from the different parental populations and both transplant sites were pooled, the discriminant analysis of plants exposed to and protected from molluscan herbivores and the control treatments revealed a great deal of overlap in the form of these plants (Table 1-6, Figure 1-5). A multivariate analysis of variance indicated a marginally significant difference between treatments (Hotellings generalized T-zero squared= 27.0599, Tiku's approximate chisquare= 16.83, DF= 8.500, p=0.0409; BMDP, Dixon 1983) but the univariate analysis of each of the variables was not significant. When analyzed separately, there was considerable overlap in the form of plants protected from molluscs but exposed to urchins (at Prasiola Low) and those protected from molluscs where urchins were absent (Prasiola Point; Table 1-7, Figure 1-6). A multivariate T-Test indicated no significant differences between these two sites (Mahalanobis D^2 = 1.2649, Hotellings T^2= 4.4975, F value= 0.5291, DF = 7, 28.0, p= 0.8049; BMDP, Dixon 1983).

THE INFLUENCE OF FORM ON DRAG

Analysis of covariance was used to determine if drag differed between plants from Bluestone and Prasiola at three different water velocities (Number Cruncher Statistical System, Hintze 1985). Surface area of the plant was considered to be the best measure of plant size (plants of the same height varied greatly in the number of branches they posessed) and was used as the covariate (Table 1-8). Plants from Bluestone had higher drag than those from Prasiola at 0.08m/s and at 0.42m/s. There was no significant difference between the two morphs at 0.27m/s. This may be due to a large variance in the values caused by a transition from laminar to turbulant flow (Wheeler in prep.).

DISCUSSION

Calliarthron changes in form along wave exposure gradients. In more exposed environments the waves are larger and more frequent, resulting in greater water velocities, greater accelerations, and therefore greater forces on organisms (Denny et al. 1985). In these environments Calliarthron plants are very highly branched and all branching occurs in the same plane. In less wave exposed environments Calliarthron plants have fewer branches and the branching is three dimensional. The plants in less wave exposed habitats are also much taller than those in more exposed areas. Drag on the plants of the two different morphotypes was as one would predict if thallus form in this species reflected adaptation to water motion and the importance of drag. Plants in the more wave exposed environments had lower drag (minimizing the risk of being dislodged) than plants in the more protected environment where greater drag would increase nutrient uptake (Wheeler 1980, Wheeler and Neuschul 1981, Wheeler in prep.).

Similar patterns of variation in structure with differing degrees of wave exposure have been observed in other species. Payri (1984) found that Turbinaria in protected lagoon areas were longer, and less branched than those on more exposed reef fronts and de Paula and de Oliveira (1982) found similar results with Sargassum. These types of branching differences associated with differing wave exposure may be a general response in many different types of branched algae.

The differences in morphology between the population of plants at a more protected site (Bluestone) and a more exposed site appear to be due to both genetic and environmental factors. However, more experiments conducted with greater numbers of replicates and conducted over a longer time period would be required to conclusively determine the role of each of these factors.

Herbivores did appear to have an influence on the structure of Calliarthron if one considers all features simultaneously in the multivariate analysis. However, there was no significant difference between plants protected from both molluscan and echinoderm herbivores and those protected from molluscan herbivores and exposed to urchins. Herbivores have been found to be important in another apparently plastic species, Padina jamacensi. Lewis et al. (1987) concluded that herbivorous parrot fish were the primary force influencing the abundance of two different Padina morphologies, a prostrate and an erect form, rather than physical conditions that had been assumed to control this in the past (Lewis et al. 1987).

Although many marine macrophytes are plastic in their structure, not all vary to the same degree; some differences in plant structure are due to genetically different ecotypes. Also, perenial plants appear to be more plastic than annual plants (Mathieson et al. 1981). The advantages of plasticity are generally linked to dispersal into different environments or to living in an environment that is likely to be variable during the lifetime of an individual (Bradshaw 1965, Palumbi 1984). If an individual maintains the capacity to respond phenotypically to changes in the environment, then its offspring will be able to do well in a variety of possible environments, thus reducing the possible cost associated with dispersal. In contrast, individuals that are particularly adapted to one environment will do well in that environment, but will be at a disadvantage if they happen to land in another environment. These two strategies are generally assumed to represent tradeoffs: the organism that specializes will do better than the generalist in the environment to which it is adapted, whereas the generalist will be capable of living in many different environments (Pianka 1974, Bradshaw 1965). However, if an organism lives in an environment that is likely to change, then the ability to be plastic would be an advantage (Palumbi 1984).

Calliarthron is long lived, and appears to have very local spore dispersal (Johansen 1969, Padilla 1981, 1984). The plasticity in Calliarthron therefore may not be adaptive for broad colonization of new enviroments, but rather for surviving in a temporally variable environment. The fact that there was no sporeling colonization at the Bluestone site during two years, but there is an established and growing adult population suggests that this environment can be locally highly variable. Also, although no reproductive plants could be found at the Prasiola Low site during the course of this study, many reproductive plants were found when the study was terminated in August of 1985.

Alternatively, dispersal may not be as local as it appears. As these plants are very long lived, rare events of longer distance dispersal (i.e., branches broken and washed to a new shore) may be very important, particularly if these happen when one would expect large scale disturbance and the opening of new habitat. Padilla (1981) found that large tide pools have little free space and are rather constant in their community composition over time. Therefore, they are difficult for new individuals to invade as the majority of space is occupied by vegetative growth and overgrowth. Therefore the ability to colonize these small amounts of space successfully when they become available could be very important. By maintaining plastic responses, the plants could successfully invade a large number of various habitats when they become available.

Calliarthron appears to have local spore dispersal, and some genetic differentiation in the differences in structure among populations. There also appears to be a phenotypic responses to the local environment. The differences in structure appear to be adaptive with respect to water motion and drag. The ability to have a flexible structure may be particularly advantageous in a variable, disturbed environment like the rocky intertidal zone.

Table 1-1. Community composition of three habitats examined.

	Prasiola Low	Prasiola Pt.	Bluestone
WAVE EXPOSURE	MORE	MORE	LESS
WIDTH OF BARNACLE ZONE	2.51m	2.30m	1.19m
COMMUNITY COMPOSITION[a] (% COVER): NUMBER OF QUADRATS	40	42	37
BARE ROCK	22.26	6.76	20.69
CORALLINE CRUST	51.38	29.98	8.63
CALLIARTHRON	4.36	8.65	4.25
TOTAL # SPECIES	26	38	21
TOTAL HERBIVORE DENSITY (#/m2)	61.68 (198.72)*	25.04	29.92
LIMPETS	4.56	13.72 (173.78)*	11.56
CHITONS	7.96	10.08	0.72
URCHINS	48.00	0.20	0
OTHER[b]	1.16	1.04	0.24
OMNIVORES[c]	0.84	1.04	0.24
PREDATORS[d]	2.56	3.36	0

*Density if juvenile limpets are included.

[a] Percent cover and densities were determined by using a 1/4m quadrat and sampling the entire tide pool or area surrounding the transplant experiments.

[b] Other species included: gastropods, Calliostoma spp., Littorina scutulata, Littorina sitkana, and isopods, Idothea spp.

[c] Omnivores included: Pugettia, and several species of polychaete worms.

[d] Predators included: Pisaster ocraceous and Lepasterias hexactus.

Table 1-2. Twoway Analysis of variance with intergenicular position and intergenicular width, and with intergenicular height and depth as covariates for 20 transplanted sporelings within the same treatment (protected from molluscan herbivores).

Source	Deg. of Freedom	Sum of Squares	F-Ratio	P
Plant	19	111.333	0.66	0.82
Intergenicular Position	10	27.684	0.31	0.97
Intergenicular Depth	1	5.823	0.65	0.51
Intergenicular Height	1	9.670	1.08	0.31
Explained	31	154.513	0.56	0.94
Error	24	214.211		
Adj. Total	55	368.723		

Test for difference in slopes, not significant (p=0.85).

Table 1-3. Means and standard deviations (in parentheses) of parental plant features used in the analysis. See Figure 1-2 for plant dimensions.

	Bluestone	Prasiola Pt.	Prasiola Low
Base Height	25.267 (19.142)	12.425 (4.510)	20.895 (8.355)
Total Height	42.501 (34.813)	23.620 (14.291)	54.055 (7.217)
Intergenicular height	2.486 (0.412)	1.912 (0.313)	2.729 (0.558)
Intergenicular width	1.738 (0.304)	2.576 (0.677)	3.368 (0.503)
Intergenicular depth	1.271 (0.148)	1.157 (0.371)	1.533 (0.276)
Intergen.wd./ Intergen.dpth.	1.391 (0.274)	2.436 (0.721)	2.139 (0.276)
Intergen.ht./ Intergen.wd.	1.515 (0.400)	0.085 (0.213)	0.835 (0.286)
Dimension of branches	3.000 (0)	2.000 (0)	2.000 (0)
Degree of branching	1.600 (0.843)	1.909 (0.944)	2.750 (0.500)
Number of Plants	10	11	3

Table 1-4. Morphological data from all experimental plants that were large enough for analysis. Table values are the Transplant Location (2= Prasiola Point, 3= Prasiola Low), the Experimental Treatment (1= with herbivores, no exclosures; 2= molluscan herbivores excluded; and 3= control for copper exclosures), the Base Height, Total Height, Intergenicualr Height, Intergenicular Width, and Intergenicular Depth.

Plants from Bluestone Parents:

Trans. Loc.	Exp. Treat	Base Ht.	Tot. Ht.	I.G. Ht.	I.G. Wd.	I.G. Depth
2	2	6.14	6.14	1.63	2.08	.71
2	2	9.36	9.36	2.11	3.04	.64
2	2	17.56	17.56	2.26	3.26	.74
2	2	7.21	7.21	1.89	1.75	.59
2	3	20.94	41.63	2.01	2.65	.96
3	1	6.87	17.21	2.01	3.54	.85
3	1	23.23	23.23	2.04	3.19	.86
3	1	13.71	13.71	2.42	2.44	.64
3	1	7.69	7.69	1.98	2.07	.48
3	1	10.05	19.41	2.08	3.39	1.02
3	1	4.24	10.66	1.74	2.44	.66
3	1	28.38	28.38	2.23	3.17	.99
3	1	16.54	16.54	1.85	2.55	.79
3	1	8.40	8.40	2.27	2.43	.58
3	1	8.78	8.78	2.79	2.49	.47
3	3	12.87	12.87	1.71	2.23	.50
3	1	8.70	11.58	2.77	2.88	.74
3	1	9.59	13.64	2.26	2.31	.61
3	1	9.36	11.39	3.07	2.92	.78
3	3	3.18	9.19	2.09	2.52	.80
3	2	11.12	11.12	2.24	2.25	.82
3	2	7.42	7.42	2.84	2.17	.80
3	2	10.66	10.66	2.19	1.86	1.02
3	2	10.31	10.31	2.19	2.71	.68
3	2	6.52	6.52	1.96	2.98	.99
3	2	6.05	6.05	2.31	2.68	.71
3	2	19.58	25.36	2.19	5.02	1.17
3	2	8.64	8.64	1.62	1.65	.91
3	2	15.59	28.14	1.74	2.80	1.05
3	2	3.72	18.92	1.48	2.31	1.23
3	2	12.50	12.50	2.09	2.44	.69
3	2	5.00	11.3[illegible]	1.77	2.35	1.01
3	3	10.16	10.16	1.57	2.27	.87
3	2	9.35	12.31	1.77	3.02	.99
3	2	4.87	9.05	1.53	2.87	.54
3	2	8.97	15.10	1.30	2.91	.70
3	2	11.22	11.22	2.48	2.35	.55
3	2	14.94	18.66	2.53	2.94	.78
3	2	10.30	10.30	1.88	2.55	.83

Table 1-4 (continued)

Plants from Bluestone Parents:

Trans. Loc.	Exp. Treat	Base Ht.	Tot. Ht.	I.G. Ht.	I.G. Wd.	I.G. Depth
3	2	21.53	21.53	2.44	2.77	.78
3	2	9.27	9.27	1.93	1.88	.90
3	2	12.19	12.19	2.47	2.55	.79
3	2	9.42	9.42	2.01	2.06	.52
3	2	14.07	14.07	2.65	3.06	.78
3	2	10.05	10.05	1.97	2.44	.76
3	2	7.26	7.26	2.67	2.54	.77
3	3	5.70	5.70	1.39	1.85	.48

Plants from Prasiola Point Parents:

Trans. Loc.	Exp. Treat	Base Ht.	Tot. Ht.	I.G. Ht.	I.G. Wd.	I.G. Depth
2	1	30.54	44.22	1.73	3.36	.93
2	1	20.52	20.52	1.78	2.91	.94
2	1	21.63	21.63	1.90	3.05	1.08
2	1	23.82	23.82	1.73	3.47	.95
2	3	23.50	42.58	1.73	2.85	.74
3	1	7.88	7.88	1.86	2.21	.51
3	1	8.17	8.17	1.93	2.10	.46
3	1	11.91	13.25	2.16	2.04	1.07
3	1	12.15	12.15	2.41	2.26	.93
3	1	3.24	13.64	2.15	1.06	.86
3	1	9.08	9.08	1.04	1.04	1.04
3	2	7.51	7.51	2.20	1.67	.65
3	2	6.06	6.60	2.96	1.50	.46
3	2	6.01	6.01	1.75	1.65	.53
3	2	12.76	12.76	2.29	2.27	.63
3	2	9.04	9.04	1.51	2.15	1.01
3	2	14.82	14.82	2.10	2.03	.90
3	2	5.91	5.91	1.37	2.19	.60
3	3	7.02	7.02	2.02	1.37	.60

Table 1-5. Means and standard deviations (in parentheses) of transplant features used in the analysis for sporelings whose parents were from either Bluestone or Prasiola Point. Table values are for plants from both Prasiola Pt. and Prasiola Low, pooled across all treatments. See Figure 1-2 for plant dimensions.

	Sporelings of Bluestone Parents	Sporelings of Prasiola Parents
Base Height	12.743 (7.644)	10.834 (5.342)
Total Height	15.085 (11.319)	13.359 (7.036)
Intergenicular height	1.926 (0.416)	2.094 (0.401)
Intergenicular width	2.165 (0.710)	2.068 (0.569)
Intergenicular depth	0.783 (0.218)	0.776 (0.184)
Intergen.wd./ Intergen.dpth.	2.986 (0.982)	3.512 (0.839)
Intergen.ht./ Intergen.wd.	1.040 (0.485)	0.857 (0.185)
Number of Plants	19	47

Table 1-6. Means and standard deviations (in parentheses) of the features used in the analysis for the experimental transplant treatments. Table values are pooled across parental populations (Prasiola Pt. and Bluestone) and transplant sites (Prasiola Pt. and Prasiola Low). See Figure 1-2 for plant dimensions.

	With Molluscan Herbivores	Without Molluscan Herbivores	Control
Base Height	13.238 (7.700)	10.096 (4.167)	11.910 (7.730)
Total Height	15.869 (8.502	11.676 (5.407)	18.450 (16.322)
Intergenicular height	2.100 (0.462)	2.604 (0.412)	1.787 (0.259)
Intergenicular width	2.577 (0.677)	2.464 (0.640)	2.247 (5.04)
Intergenicular depth	0.791 (0.205)	0.783 (0.188)	0.705 (0.186)
Intergen.wd./ Intergen.dpth.	3.472 (1.022)	3.300 (0.875)	3.322 (0.785)
Intergen.ht./ Intergen.wd.	0.905 (0.319)	0.921 (0.296)	0.868 (0.282)
Number of Plants	23	36	7

Table 1-7 Means and standard deviations (in parentheses) of the features used in the analysis for comparing the without molluscan herbivore treatments between Prasilola Pt. (no urchins) and Prasiola Low (urchins present). Tabled values are from sporelings from both parental populations pooled. See Figure 1-2 for plant dimensions.

	Prasiola Point No Urchins No Molluscs	Prasiola Low Urchins Present No Molluscs
Base Height	10.068 (5.171)	10.100 (4.131)
Total Height	10.068 (5.171)	11.887 (5.482)
Intergenicular height	1.970 (0.276)	2.076 (0.428)
Intergenicular width	2.530 (0.732)	2.456 (0.641)
Intergenicular depth	0.669 (0.067)	0.798 (0.194)
Intergen.wd./ Intergen.dpth.	3.796 (0.979)	3.273 (0.857)
Intergen.ht./ Intergen.wd.	0.820 (0.183)	0.933 (0.307)
Number of Plants	4	32

Table 1-8. One-Way Analysis of covariance table for drag, with plant surface area as a covariate, for the two morphotypes of Calliarthron from Bluestone and Prasiola.

For 0.08m/s Water Velocity:

Source	DF	Sum of Squares ($\times 10^{-3}$)	F-Ratio	Tail Probability
S. Area	1	0.0622	14.96	0.00
Site	1	0.0046	1.12	0.30
Explained	2	0.0669	8.04	0.00
Residual	29	0.1206		
Total	31	0.1875		

Mean Drag: Bluestone- 0.0370N
Prasiola- 0.0358N

For 0.27m/s Water Velocity:

Source	DF	Sum of Squares ($\times 10^{-3}$)	F-Ratio	Tail Probability
S. Area	1	0.0811	1.05	0.31
Site	1	0.2590	3.36	0.07
Explained	2	0.3400	2.20	0.13
Residual	29	2.2365		
Total	31	2.5766		

Mean Drag: Bluestone- 0.0817N
Prasiola- 0.0595N

For 0.42m/s Water Velocity:

Source	DF	Sum of Squares ($\times 10^{-3}$)	F-Ratio	Tail Probability
S. Area	1	0.9754	1.79	0.19
Site	1	3.9153	7.17	0.01
Explained	2	4.8907	4.48	0.02
Residual	29	15.8397		
Total	31	20.7304		

Mean Drag: Bluestone- 2.0648N
Prasiola- 1.8727N

Figure 1-1. Map of Barkeley Sound and study sites.

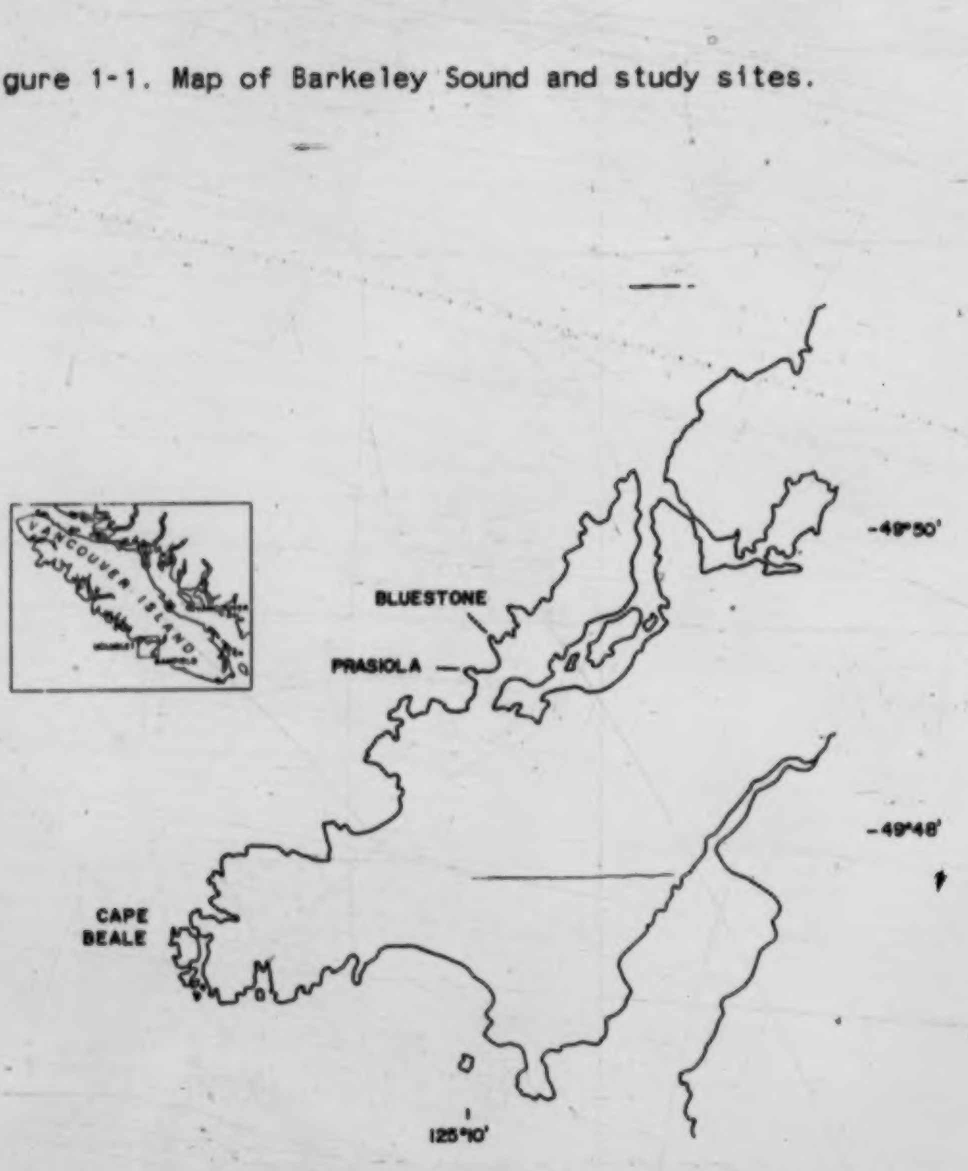

VANCOUVER ISLAND
BLUESTONE
PRASIOLA
CAPE BEALE
-49°50'
-49°48'
125°10'

Figure 1-2. Diagram of Calliarthron. The morphometric measurements that were taken are indicated.

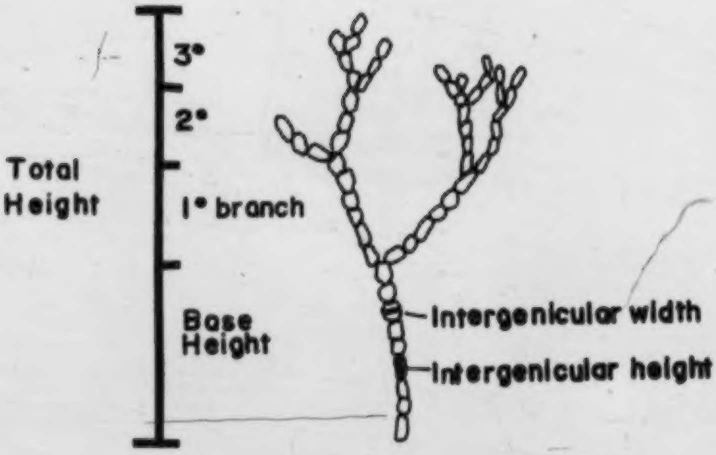

3°
2°
Total Height
1° branch
Base Height
Intergenicular width
Intergenicular height

Figure 1-3. Histogram of canonical variables for parental plants. b=Bluestone, p=Prasiola Pt., and l=Prasiola Low.

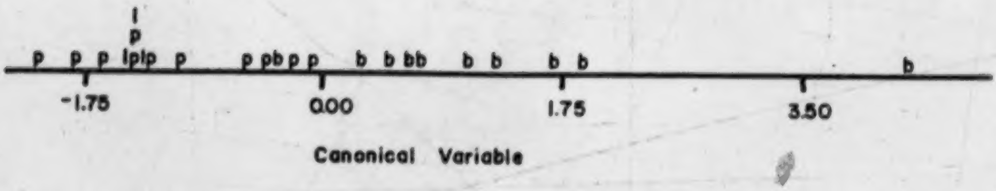


Figure 1-4. Plot of canonical variables for all transplant and parental plants. A(1)=Bluestone parents. B(2)=Prasiola parents. C(3)=Transplants from Prasiola. D(4)=Transplants from Bluestone.

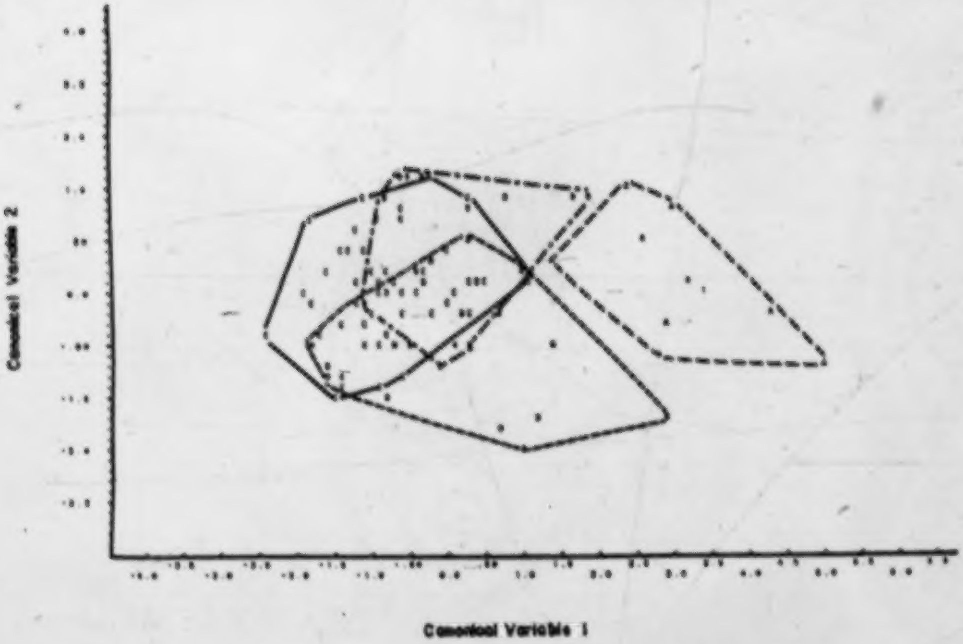


Figure 1-5. Plot of canonical variables for each of the three experimental treatments, with herbivores=A(1), without herbivores=B(2), and controls=C(3).

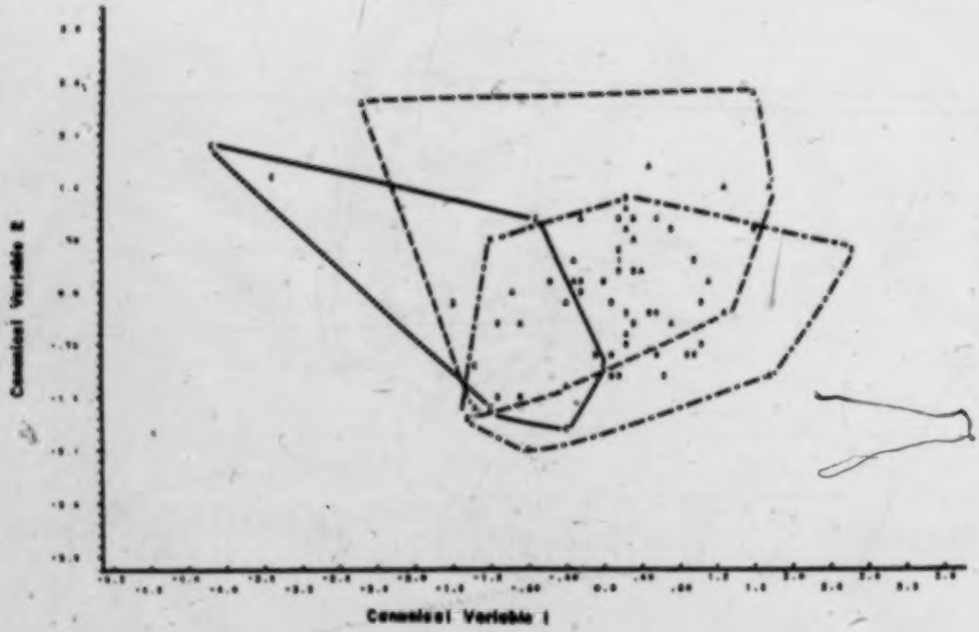
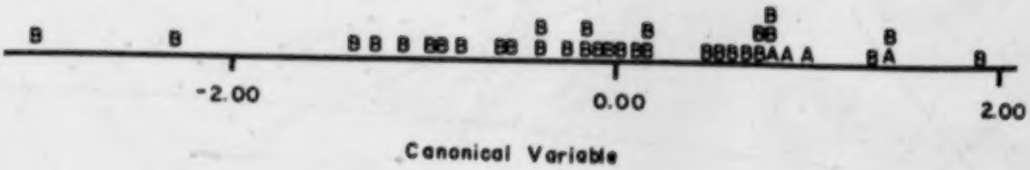


Figure 1-6. Histogram of canonical variable for transplants protected from molluscs but either exposed to urchins (Prasiola Low) or not exposed to urchins (where urchins do not occur, Prasiola Point).



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